The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

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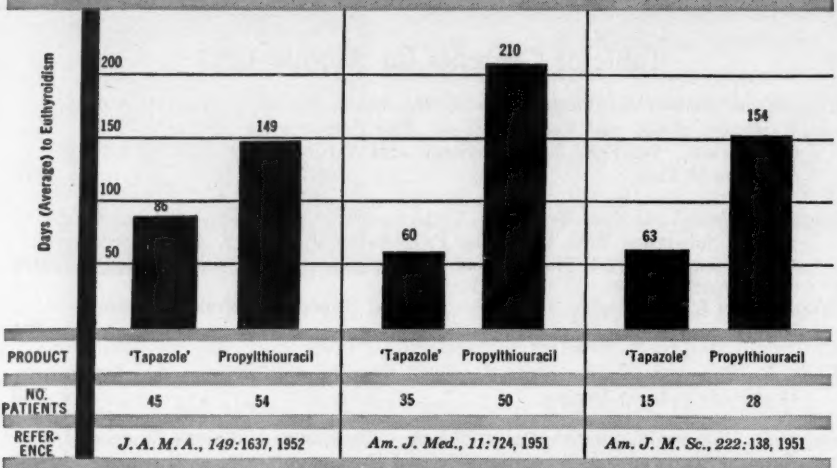

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VOLUME 15 AUGUST, 1955 NUMBER 8
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THE COMPARATIVE EFFECTS OF METACORTANDRACIN, 9-α-FLUOROHYDROCORTISONE AND HYDROCORTISONE UPON ACTH SECRETION IN MAN*

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WILKINS first reported on the efficacy of cortisone and related compounds in depressing excessive 17-ketosteroid excretion in patients with the adrenogenital syndrome (1–6). These observations were promptly and extensively confirmed by others (7–19). Kupperman *et al.* suggested that the degree of depression of 17-ketosteroids is quantitatively related to the dose of corticoid employed (16). These investigators demonstrated that the suppressive effect of cortisone upon 17-ketosteroid excretion could be employed experimentally to determine the relative activity of different cortisone derivatives (15). This method also proved to be an effective technic in establishing the relative activity of different steroid combinations other than the corticoids alone in depressing 17-ketosteroid excretion (15). Since the effect of the corticoids upon the 24-hour 17-ketosteroid excretion is actually an indication of their ability to inhibit ACTH secretion, the 17-ketosteroid levels observed after cortisone therapy reflect the degree of ACTH inhibition that has been achieved by these

* Presented at the Annual Meeting of The Endocrine Society, Atlantic City, N. J., June 2–4, 1955.

compounds. The following report is concerned with the comparative effect of metacortandracin[1] (21–22), 9-α-fluorohydrocortisone, hydrocortisone, and cortisone upon inhibition of ACTH secretion from the pituitary gland of patients with adrenal hyperplasia, as measured indirectly by the changes observed in 17-ketosteroid excretion.

PROCEDURE

The effect of various corticoids upon ACTH secretion in the human was evaluated in a series of well documented patients with the adrenogenital syndrome, by determining the degree of inhibition of excessive 17-ketosteroid excretion. All the patients were females and usually exhibited some or all of the classic manifestations of increased endogenous androgenic secretion, as characterized by amenorrhea or menstrual abnormalities, increased hirsutism, clitoral enlargement, and male-like habitus. One patient with normal adrenocortical function was also studied. The steroid to be evaluated was administered orally to ambulatory patients for a period of at least one week and many times over a period of several months. Twenty-four-hour urine samples were collected at the prescribed intervals. The patients were instructed and strongly impressed with the necessity for the accurate collection of these 24-hour samples. A modification of the method of Drekter *et al.* was used for the 17-ketosteroid determinations (20). The dose of cortisone or its derivatives was varied according to the depression of 17-ketosteroid excretion that was achieved. In addition to noting the ability of the cortisone derivatives to suppress urinary 17-ketosteroids, the clinical effectiveness of the preparations was also observed. The clinical response in the patients was judged by the establishment of regularity of menstrual periods, induction of ovulation, increase in breast size, decrease in rate of hair growth and development of a more feminine build.

The usual doses of the cortisone and derivatives that were employed in these studies were as follows: Cortisone was administered in doses ranging from 10.0 mg. to 25.0 mg. twice to three times per day, depending on the extent of depression of 17-ketosteroid excretion that was attained. Hydrocortisone was administered usually in doses of 10 mg. two to three times a day. 9-Alpha-fluorohydrocortisone was administered in doses of 1 mg. two to three times a day; and metacortandracin was administered in doses ranging from 5 mg. twice a day to 2.5 mg. three times or twice a day. Frequently the patients were changed from one corticoid to another, the type and dose of preparation being changed arbitrarily. However, an attempt was usually made to administer the dose of corticoid necessary to maintain 17-ketosteroid excretion at least between 10 and 15 mg. or less per twenty-four hours. Alteration of the dose of various preparations was based, not upon demonstrable changes in clinical status, but upon the 17-ketosteroid levels.

RESULTS AND DISCUSSION

Values on individual patients are shown in Table 1. The average results obtained are depicted in Figure 1. The 17-ketosteroid excretion levels in four characteristic studies are shown graphically in Figures 2–5. From these data it is apparent that, on a weight basis, singular differences in activity exist between the different corticoids tested. The variability

[1] The generic name of metacortandracin has recently been changed to prednisone.

TABLE 1. EFFECT OF DIFFERENT CORTICOIDS UPON URINARY 17-KETOSTEROID EXCRETION IN THE ADRENOGENITAL SYNDROME

Patient			Urinary 17-ketosteroid excretion (mg./24 hrs.)													
Name	Age (yrs.)	Amenorrhea	Before treatment	*Cortisone*				*Hydrocortisone*				*Metacortandracin*			*9-α-Fluorohydrocortisone*	
				50*	37.5	25	20	40	30	25	20	10	7.5	5	3	2
S.A.	29	2°	19.8		11.5				4.8 5.5		11.6	4.2	5.6 5.6		5.9	
J.H.	22	2°	34.0 32.0						9.2			9.1 10.3	7.5			
S.F.	29	2°	36.0 29.0						22.1			20.3	24.0 29.0			
G.O.	29	1°	78.0		11.6 15.9		19.0		7.0	7.5	8.3 7.3	6.8	4.9	7.5		7.2
M.M.	16	1°	58.0 79.5 81.8		35.1				29.2 27.2			20.2			11.7	
P.F.	18	1°	72.5 56.4 69.0					27.1	25.6			30.0 14.8	20.2 18.9			
A.B.	21	2°	22.0								8.4 9.7	5.3 11.0 5.5				
M.L.	24	2°	19.8 20.0		8.4							8.9	9.2			
L.A.	18	1°	60.0	12.6								11.2 11.3	16.1			
S.K.	27	Olig.	23.0		15.6				8.8	16.8		10.6 14.7				
R.F.	27	2°	40.2		26.5 13.1 19.2	17.9			14.5 6.3 9.7			8.6				
R.B.	21	2°	32.0		12.0				9.0 11.5	18.6		13.5 8.6	17.0			

* Daily dose in milligrams.

in the 17-ketosteroid excretion, although admittedly large, was not excessive in the individual patient.

The data obtained in general bear out the concept that comparative activity of the different cortisone derivatives may be ascertained by this experimental procedure (15–16). It is interesting to note that when the doses of cortisone or derivatives were reduced, there was a corresponding increase in excretion of 17-ketosteroids. However, it may be said that the doses employed in all the patients were comparatively small and maximal adrenocortical inhibition was not achieved.

The possibility that the administered adrenal steroid itself was excreted as a ketosteroid and thereby partly account for some of the 17-ketosteroid values obtained can be discounted on the basis of our past experiences. Thus patients in this institution who had previously received 1,000 mg. of cortisone per day for the control of dermatosis showed slight, if any,

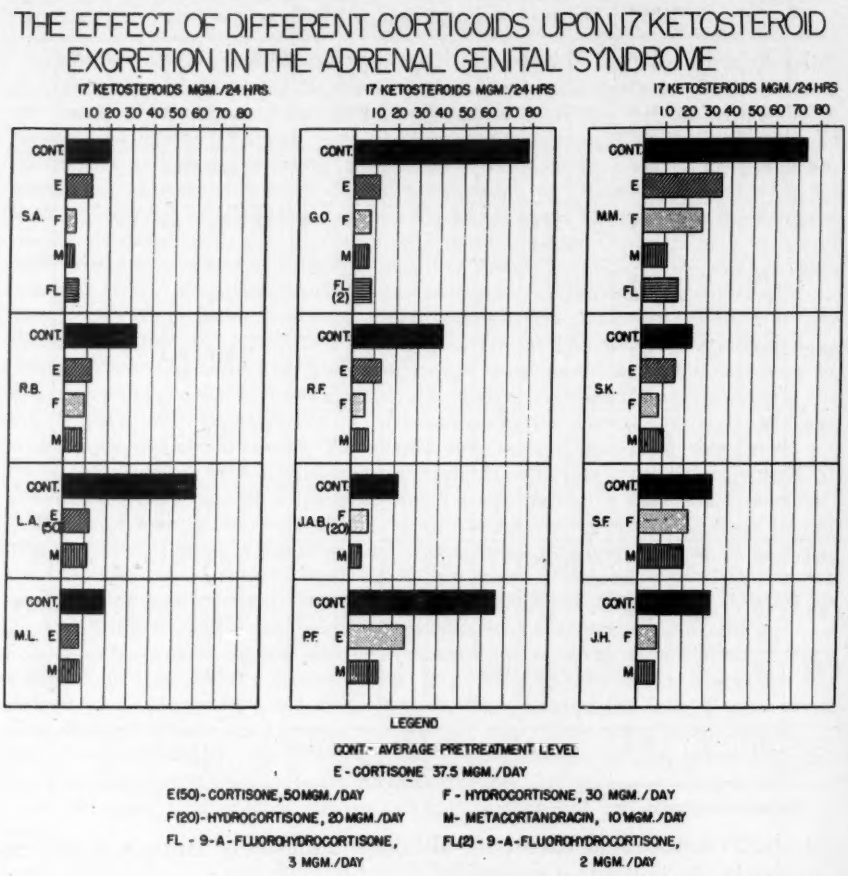

FIGURE 1

increase in ketosteroid excretion over that noted during their control pretreatment periods (23).

One normal patient with rheumatoid arthritis showed a depression of 17-ketosteroids when she received 5 mg. of metacortandracin three times a day, a dose 50 per cent greater than the maximum dose of this preparation used in patients with adrenal hyperplasia. Her urinary 17-ketosteroids

decreased from 13 mg. to 5.5 mg. after three weeks of therapy. It is to be noted, however, that complete amelioration of an acute rheumatoid episode was accomplished with this regimen.

The doses administered in the entire study of patients with the adrenogenital syndrome were usually less than those employed in the treatment of the dermatoses or rheumatoid arthritis. Toxicity was not recorded with any of the preparations or doses employed, except 9-α-fluorohydrocortisone. The toxicity observed with this preparation was manifested by fluid and sodium retention, characterized by dependent and periorbital edema. The patients who became toxic also complained of severe headaches and joint pains. Six patients were originally given 1 mg. of the

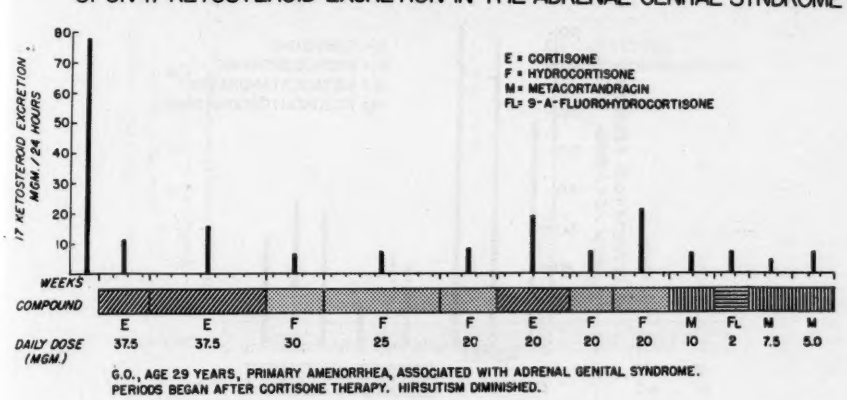

FIGURE 2

steroid three times a day; in 5, the foregoing toxic symptoms developed. These reactions often necessitated discontinuing the medication and prevented fully adequate study of the drug. Three patients, however, continued on oral 9-α-fluorohydrocortisone for a sufficiently long period of time to permit preliminary 17-ketosteroid studies to be made. From the data, it was felt that although the dose of 9-α-fluorohydrocortisone was effective in suppressing 17-ketosteroid excretion (Table 1 and Figures 1–3), it was not suitable of further clinical trial in patients with the adrenogenital syndrome due to the extensive salt and water retention it induced. The abnormalities of water and electrolyte balance that occurred after 9-α-fluorohydrocortisone therapy were too great to compensate for its marked effectiveness in suppressing the excessive 17-ketosteroid excretion of patients with adrenal hyperplasia. One patient maintained on the same

dose after salt retention was achieved, noted some disappearance of edema and occurrence of diuresis, even though medication was continued. It should be noted that all evidence of toxicity subsided when therapy was discontinued.

On the weight basis, it can be said that of all the corticoids employed, 9-α-fluorohydrocortisone was the most potent with respect to pituitary ACTH inhibition and can be considered as being some 10 times as active as hydrocortisone and 12 to 13 times as active as cortisone. On the other hand, metacortandracin was estimated as being three times as active as

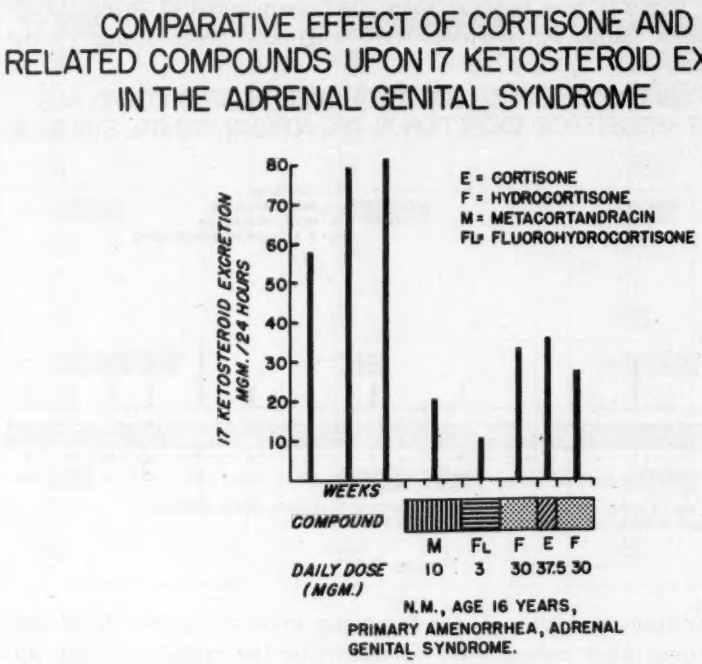

FIGURE 3

hydrocortisone and some 3 to 4 times as active as cortisone. In a study of the activity of these steroids, based upon a comparison of our 17-ketosteroid excretion values with those reported by other workers, we found that our results were in approximate agreement with the estimations made in studies of patients who received these drugs for the control of rheumatoid arthritis. In the rheumatoid group of patients, it has been estimated that hydrocortisone is some 25 per cent more active in controlling the symptoms of rheumatic fever, as compared to cortisone itself. It has also been noted that metacortandracin is about 4 to 5 times as active

as cortisone, or 3 to 4 times as active as hydrocortisone, in controlling the symptoms of the rheumatoid arthritic (21).

Quantitative measurement of the degree of 17-ketosteroid inhibition indicates that metacortandracin is the most potent, nontoxic corticoid (on a weight basis) capable of inhibiting ACTH secretion in man. However, the therapeutic efficacy of all the drugs used was comparable and they all achieved depression of 17-ketosteroids associated with the same degree of clinical improvement, providing an adequate dose of the corticoid was administered. Clinical improvement was comparable after equivalent

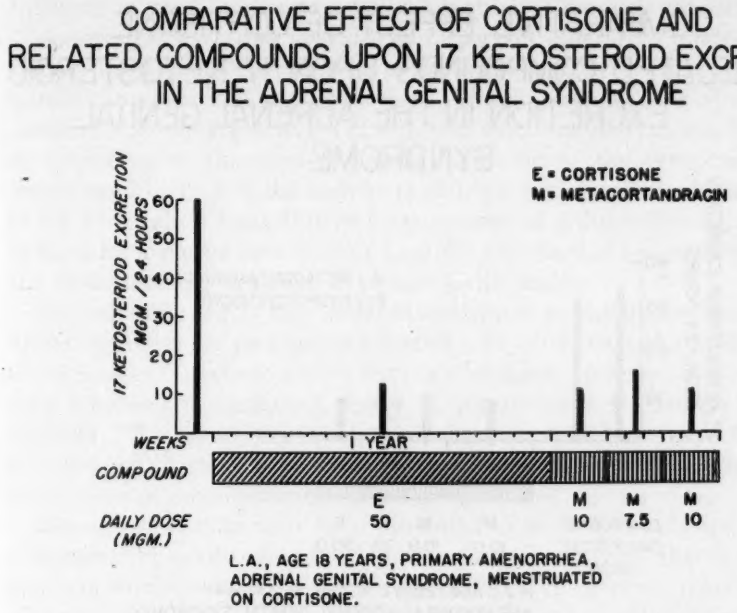

FIGURE 4

doses of the corticoid and was characterized by resumption of regular menstrual periods, increased rate of breast growth, and diminution of the evidence of excessive androgenicity.

It is interesting that in our patients, as a group, we always obtained predictable and effective results with oral administration of the corticoids, making it unnecessary to administer any of the corticoid derivatives parenterally. If suppression of the ketosteroid excretion is not achieved with the average dose noted previously, it will usually be accomplished by increasing the dose of the corticoid of choice. The average dose of cortisone advised in this clinic, based upon these and other studies, is 37.5 mg. per day. This compares to an average dose of 30 mg. per day of

hydrocortisone and to 7.5 to 10 mg. per day of metacortandracin. Although 3 mg. per day of 9-α-fluorohydrocortisone provides effective control of the patient with adrenal hyperplasia, it cannot be considered as an average clinical dose, since toxic effects were achieved with this dosage.

The mechanism of action of the corticoids in preventing the excessive urinary 17-ketosteroids in patients with the adrenogenital syndrome is based primarily upon the concept of endogenous production of hydrocortisone by the adrenal (5, 12, 13, 14). In essence, the metabolic pathways involving hydrocortisone secretion from the adrenal cortex in the

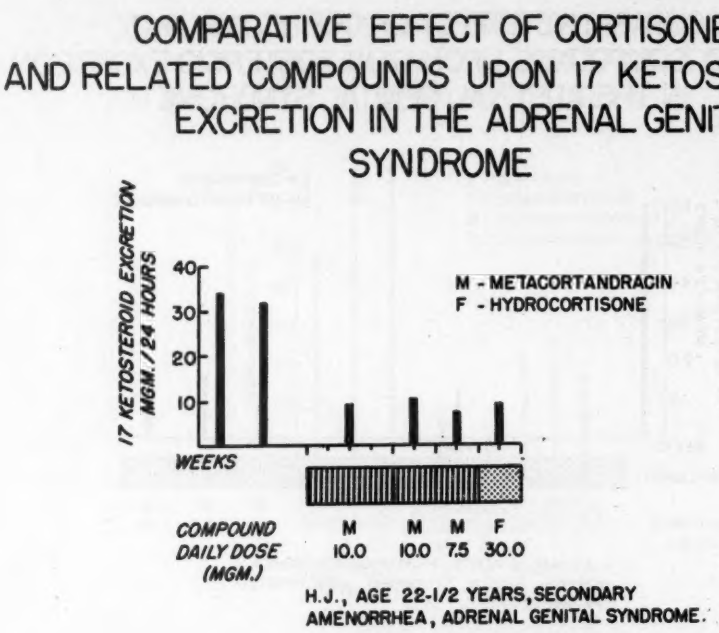

FIGURE 5

normal individual include the transformation of cholesterol to pregnanolone to progesterone to 17-hyroxyprogesterone. The 17-hydroxyprogesterone, by virtue of an enzymatic process, is converted to hydrocortisone by means of hydroxylation occurring at C-11 and C-21 (13, 14). Patients with the adrenogenital syndrome presumably have a defect in the enzymatic conversion of 17-hydroxyprogesterone to hydrocortisone, based upon the failure of hydroxylation to occur at C-11 and/or C-21, thereby interrupting the sequence of developmental transformation of cholesterol to hydrocortisone. As a result of the failure of the adrenal to secrete hydrocortisone-like derivatives, ACTH secretion from the pituitary gland

of these patients is uninhibited and increased (25). The increased ACTH secretion induces adrenal hyperplasia with increased secretion of androgenic precursors from the adrenal cortex. These, in turn, are excreted in part as 17-ketosteroids. The ACTH secretion which would normally be regulated by endogenously secreted corticoids may then be adequately achieved by the exogenous administration of the corticoids described here (5, 12). This results in the depression of the virilizing substances from the adrenal gland to normal levels, accompanied by a decrease in 17-ketosteroid excretion. This is followed promptly by clinical improvement in terms of more adequate ovarian function. Therefore, the decrease in 17-ketosteroid excretion provides an important laboratory guide in determining the relative effectiveness of corticoid therapy in the patient with adrenal hyperplasia. Conversely these patients serve well for comparative study of the relative activity of different cortisone derivatives, by virtue of the effect of the corticoid upon inhibition of the excessive ACTH secretion (25). Due to the high level of urinary 17-ketosteroids, it is much easier to obtain a quantitative measurement of a dose-effect relationship in the adrenogenital patient than to study the effect of the corticoids upon the 17-ketosteroid excretion in normal individuals.

Metacortandracin is now under investigation in patients with ovulation defects whose chief problem is infertility. It is felt that the utilization of smaller doses of a corticoid not having a tendency to induce salt retention may represent a significant degree of improvement in therapy of these patients. The same degree of adrenocortical inhibition expected after cortisone or hydrocortisone therapy would be anticipated when comparable doses of metacortandracin are administered.

In conclusion, data have been presented to show that patients with the adrenogenital syndrome are adaptable for the study of the suppressive action of cortisone and its derivatives upon ACTH secretion in man. Whatever the mechanism of action of cortisone in decreasing the 17-ketosteroid excretion may be, quantitative appraisal of the effect of the active corticoids in decreasing the 17-ketosteroid excretion is established. The activity of a specific adrenocortical preparation may now be established accurately in the human by denoting the potentiality of the steroid in suppressing the excessive 17-ketosteroid excretion in patients with the adrenogenital syndrome. These data indicate that metacortandracin is the most active, nontoxic corticoid available at the present time in the therapy of patients with adrenal hyperplasia.

SUMMARY

Data concerning the effects of hydrocortisone, cortisone, metacortandracin, and 9-α-fluorohydrocortisone in the adrenogenital syndrome have

been presented. Depression of the excessive 17-ketosteroid excretion was noted with all four preparations. 9-Alpha-fluorohydrocortisone appeared to be the most potent; metacortandracin next, followed by hydrocortisone; then cortisone, which was least active on the basis of the weight of the required dose.

9-Alpha-fluorohydrocortisone in the doses employed, readily induced toxicity, characterized by fluid retention and edema. These toxic effects make it impractical to use this corticoid in the therapy of patients with the adrenogenital syndrome.

With the exception of the foregoing toxic reactions, the overall effects of comparable doses of the described corticoids were similar. Adequate suppression of ketosteroid excretion and clinical management of the adrenogenital patient were easily accomplished by oral preparations. Clinical improvement as manifested by establishment of menstrual periods or regularity of menstrual periods, increase in breast size, and decrease in hair growth also followed the administration of each of these corticoids.

Due to the relatively low toxicity of metacortandracin and its reported lack of effect in inducing sodium retention, it is felt that metacortandracin may be the preparation of choice in treating patients with the adrenogenital syndrome or with ovulatory defects due to adrenocortical hyperplasia.

Acknowledgments

Metacortandracin was kindly supplied by Dr. N. L. Heminway of the Department of Clinical Investigation, Schering Corporation.

9-Alpha-fluorohydrocortisone was generously made available by Dr. Gilbert M. Bayne, Medical Division of Sharp and Dohme.

Cortisone and hydrocortisone were kindly supplied by Dr. H. F. Hailman of the Department of Clinical Investigation of the Upjohn Company.

Appreciation is expressed for assistance rendered by the Misses Gwendolyn Newhook and Martha Yoza, research nurses, Department of Therapeutics, New York University-Bellevue Medical Center.

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17-ALPHA-HYDROXYPROGESTERONE-CAPROATE: A NEW SUBSTANCE WITH PROLONGED PROGESTATIONAL ACTIVITY. A COMPARISON WITH CHEMICALLY PURE PROGESTERONE*

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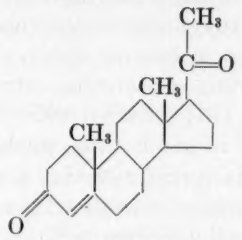
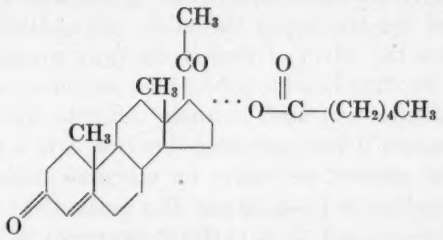
IT IS the purpose of this paper to compare the progestational activity of a new substance, 17-alpha-hydroxyprogesterone-17-caproate, with the activity of chemically pure progesterone.



Progesterone
($C_{21}H_{30}O_2$ mol. weight 314.45)



17-alpha-hydroxyprogesterone-17-caproate
($C_{27}H_{40}O_4$ mol. weight 418.51)

17-Alpha-hydroxyprogesterone-caproate is of interest because of the following factors:

1. 17-Alpha-hydroxyprogesterone, without esterification, has no progestational activity (1, 2).

2. The 17-ester (caproate or acetate), however, shows marked progestational activity in animal experiments (1).

3. Esterification of sex hormones generally causes a prolongation and, to some extent, an increase of their effects. The character of these effects (inert to highly active), however, is not changed by esterification (3, 4). 17-Alpha-hydroxyprogesterone seems to be an exception to this general rule.

4. 17-Alpha-hydroxyprogesterone is not metabolized to pregnanediol in the human (5).

5. The metabolism of 17-alpha-hydroxyprogesterone and the metab-

Received for publication January 19, 1955.

* This work has been carried out under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

olism of 17-alpha-hydroxyprogesterone-caproate in the human female are not yet fully established. There is only one report by Langecker and Prescher (6), who found that in a male patient the excretion of pregnanediol was decreased after the administration of 17-alpha-hydroxyprogesterone.

Boschann (7) showed in an endometrial study on castrates that 17-alpha-hydroxyprogesterone caproate has progestational activity. He recommended the administration of this steroid in two dosages.

MATERIALS AND METHOD

In the present study, a single injection of 17-alpha-hydroxyprogesterone-caproate was administered to each of 3 castrated women. Two weeks prior to its administration, each patient received an injection of estradiol-valerate. We used estradiol-valerate instead of estradiol-benzoate, the material used by Boschann (7), to induce proliferation of the endometrium, and we compared the effect of 17-alpha-hydroxyprogesterone-caproate with the effect of chemically pure progesterone in control observations.

To stimulate growth of the inactive endometrium of castrates, estradiol-valerate was used in three different dosages. This estrogen was chosen because it has a prolonged action over a period of about three weeks (8). The amount necessary for adequate endometrial proliferation is a single injection of 15 to 20 mg. If a single dose of 20 mg. of estradiol-valerate is administered to a castrate, estrogen withdrawal bleeding will occur in twenty days.

In order to escape the possible occurrence of menstruation-like bleeding in this study (which would be due only to the regression of estrogenic effect —estrogen withdrawal bleeding—rather than to progesterone withdrawal bleeding) an appreciable amount of estradiol-valerate (5, 10, 20 mg.) was administered with the 17-alpha-hydroxyprogesterone-caproate or with the chemically pure progesterone, respectively.

A relatively high dosage for both the progestational substances was chosen, in order to show whether or not the prolonged action could be correlated with the size of the dose.

RESULTS

1a. *17-α-OH-progesterone-caproate*

Procedure:

20 mg. of estradiol-valerate on the first day.

20 mg. of estradiol-valerate and 350 mg. of 17-alpha-hydroxyprogesterone-caproate on the fourteenth day.

Curettage at the onset of bleeding.

An interval of at least a month without hormonal therapy.

Clinical results: The uterine bleeding occurred twenty-eight days after the first injection (20 mg. of estradiol-valerate), and fourteen days after the second injection.

Endometrial histology: The glands were greatly dilated and showed a variation from a secretory type of cell lining to a nonsecretory pattern. The picture appeared to be that of an excessive stimulation of the endometrium which had produced a cystic hyperplasia in a secretory-type of endometrium.

Vaginal cytology: The vaginal smears showed a marked estrogenic effect after the first injection. After the second injection, the estrogenic effect was markedly reduced, although the same amounts of estrogens were given.

The onset of the bleeding was apparently influenced by the progestational activity rather than by estrogen withdrawal. Assuming that an identical second estrogenic dosage in the pure-progesterone control would produce even more hyperplastic and less secretory endometrium, the second dosage of estrogens was reduced in the control.

1b. *Control—Progesterone*

Procedure:

20 mg. of estradiol-valerate the first day.

5 mg. of estradiol-valerate and 350 mg. of chemically pure progesterone (oil solution) on the fourteenth day.

Curettage at the onset of bleeding.

Clinical results: The uterine bleeding occurred much earlier than in the first experiment—on the seventh day after the mixed injection or on the twenty-first day after the first injection.

Endometrial histology: The histologic sections showed glandular hyperplasia without secretory changes.

Vaginal cytology: There were no luteal-like smears found after the injection of pure progesterone.

2a. *17-α-OH-progesterone-caproate*

Procedure:

20 mg. of estradiol-valerate on the first day.

10 mg. of estradiol-valerate and 350 mg. of 17-alpha-hydroxyprogesterone-caproate on the fourteenth day.

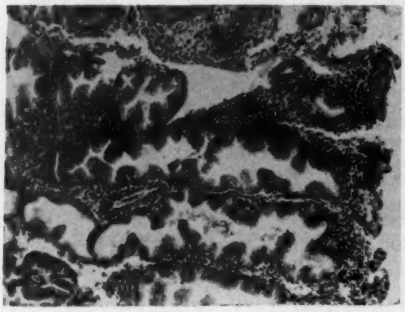
Curettage at the onset of bleeding.

An interval of at least a month without hormonal therapy.

In this patient (who received with the second injection only 10 mg. of estradiol-valerate, both in the 17-alpha-hydroxyprogesterone-caproate study and in the control study with chemically pure progesterone) the

results are more significant, as we have the identical estrogenic dosage in both studies.

Clinical results: Uterine bleeding started on the nineteenth day after the injection of 10 mg. of estradiol-valerate and 350 mg. of 17-hydroxy-



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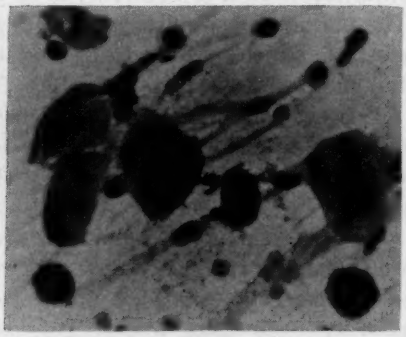

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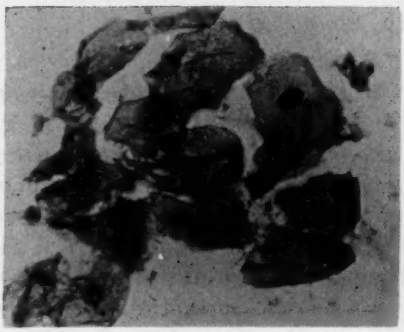

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FIG. 1. Histologic section of endometrium from castrated woman (Case 2), nineteen days after injection of 17-alpha-hydroxyprogesterone-caproate, showing secretory changes of the endometrium.

FIG. 2. Vaginal smear of a castrated woman (Case 2), prior to therapy, showing squamous epithelial cells of the parabasal layers, leukocytes and erythrocytes (atrophic menopausal type).

FIG. 3. Vaginal smear of the same patient(Case 2) thirteen days after administration of 20 mg. of estradiol-valerate, showing three superficial cornified squamous epithelial cells (marked estrogenic effect).

FIG. 4. Vaginal smear of the same patient (Case 2) four days after the simultaneous administration of 10 mg. of estradiol-valerate and 350 mg. of 17-alpha-hydroxyprogesterone-caproate and eighteen days after the first injection of 20 mg. of estradiol-valerate, showing crowded and folded intermediate or superficial non-cornified squamous epithelial cells with slight cytolytic changes (luteal effect).

progesterone-caproate, or on the thirty-third day after the first injection of 20 mg. of estradiol-valerate.

Endometrial histology: Numerous large fragments of endometrium in which the glands showed the irregularity in contour and the increase in size of individual cells characteristic of secretory endometrium (Fig. 1).

Vaginal cytology: After the injection of 17-alpha-hydroxyprogesterone-caproate the smear changed to a more luteal-like pattern.

The cytologic findings were in this case especially striking, because,

FIG. 5. Histologic section of endometrium of castrated woman (Case 3) fifteen days after administration of 17-alpha-hydroxyprogesterone-caproate, showing characteristic secretory changes of the endometrium.

prior to the therapy, this patient had a typical atrophic menopausal type of smear (Figs. 2, 3 and 4).

2b. *Control—Progesterone*

Procedure:

20 mg. of estradiol-valerate on the first day.

10 mg. of estradiol-valerate and 350 mg. of pure progesterone in oil solution on the fourteenth day.

Curettage at the onset of bleeding.

Clinical results: Uterine bleeding began on the eighth day after the

injection of 350 mg. of pure progesterone in oil solution together with 10 mg. of estradiol-valerate, or twenty-two days after the first injection of 20 mg. of estradiol-valerate.

Endometrial histology: The endometrium exhibited cystic hyperplasia of the glands.

Vaginal cytology: There was no appreciable regression to a lower type of proliferation, or to a luteal-like pattern.

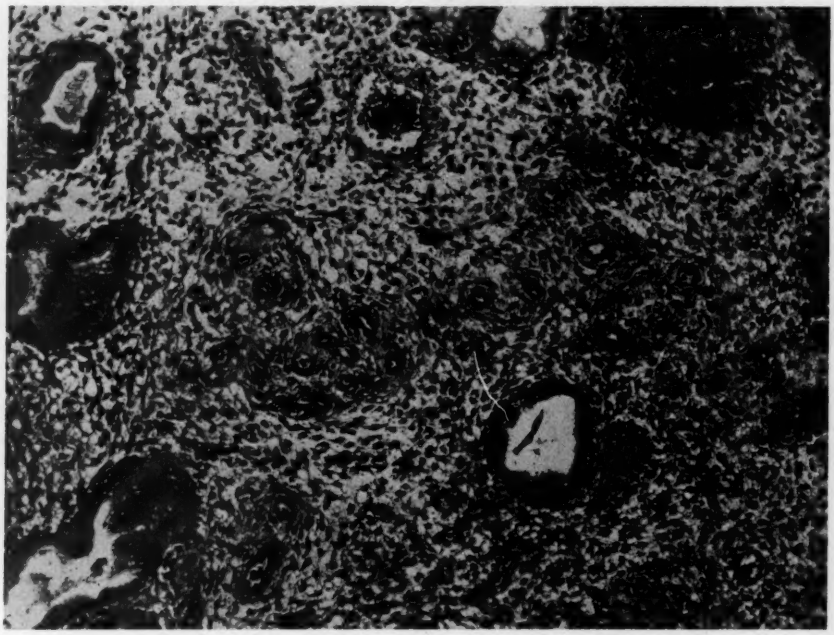

FIG. 6. Histologic section of the endometrium in the same patient (Case 3) under higher magnification, showing the spiral arteries characteristic of secretory endometrium.

3a. *17-α-OH-progesterone-caproate*

Procedure:

20 mg. of estradiol-valerate on the first day.

5 mg. of estradiol-valerate and 350 mg. of 17-alpha-hydroxyprogesterone-caproate on the fourteenth day.

Curettage at the onset of bleeding.

An interval of at least a month without hormonal therapy.

Clinical results: In this castrate, uterine bleeding started fifteen days after the injection of 17-alpha-hydroxyprogesterone-caproate and twenty-nine days after the first injection of 20 mg. of estradiol-valerate.

Endometrial histology: On histologic examination (Figs. 5, 6), a typical secretory change of the endometrium was observed.

Vaginal cytology: A characteristic luteal phase was present, a condition which is rare in exfoliative cytology for a patient under hormonal therapy.

3b. *Control—Progesterone*

Procedure:

20 mg. of estradiol-valerate on the first day.

5 mg. of estradiol-valerate and 350 mg. of pure progesterone (oil solution) on the fourteenth day.

Curettage at the onset of bleeding.

Clinical results: A menstruation-like uterine bleeding started six days after the injection of pure progesterone and estradiol-valerate, or twenty days after the first injection of 20 mg. of estradiol-valerate.

Endometrial histology: The endometrial biopsy specimen consisted of tiny fragments of endometrium. The glands had a variable pattern. Some of the fragments showed no evidence of secretion in the cells, whereas in other areas there were scattered cells in which secretory activity was visible.

Vaginal cytology: The vaginal epithelium showed a marked estrogenic effect until the eighteenth day; after that, there was a slight regression.

The marked secretory changes in the endometrium after a single administration of 17-alpha-hydroxyprogesterone-caproate are even more remarkable if we recall that this material was always injected in the first part of the experiment. The endometrium, becoming more sensitive to progestational stimulation than before, exhibited in the second part of the experiment a lesser degree of response to the administration of pure progesterone than that shown after the initial dosage of 17-alpha-hydroxyprogesterone-caproate. In other words, 17-alpha-hydroxyprogesterone-caproate was more effective than pure progesterone, over a much longer period of time, and even under less favorable circumstances.

SUMMARY

The effects of 17-alpha-hydroxyprogesterone-caproate and of pure progesterone in oil solution, injected in single doses of 350 mg. each, were compared in 3 castrated women by means of endometrial biopsies and vaginal smears. Proliferation of the endometrium was induced by an initial injection of estradiol-valerate. This estrogen was added also to the injections of 17-alpha-hydroxyprogesterone-caproate or of progesterone, respectively.

The action of 17-alpha-hydroxyprogesterone-caproate was definitely prolonged; uterine bleeding from a secretory endometrium occurred four-

teen to nineteen days after administration. In the control observations with pure progesterone, on the other hand, uterine bleeding from a practically nonsecretory endometrium occurred much earlier—about a week after administration.

Acknowledgment

We wish to express our appreciation to Dr. Edith L. Potter, of this department, for her interest and valuable suggestions. The hormones mentioned in this paper were generously supplied by E. R. Squibb & Son, New York, and Schering AG, West-Berlin, Germany.

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FAMILIAL HYPOGONADOTROPIC EUNUCHOIDISM

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AN ISOLATED deficiency of pituitary gonadotropin is rare. Familial occurrence of this form of hypogonadism has been recorded infrequently and only in male siblings (1). In some reports of familial eunuchoidism it is not possible to determine whether the defect lies in the pituitary or the gonad, since many of these cases were studied prior to the introduction of gonadotropin assays in clinical practice (2–6).

We have had the opportunity to study 5 instances of hypogonadotropic eunuchoidism in two families. Three male siblings of one family and a brother and sister of another family are the subjects of this report.

CASE REPORTS

Family I

Case 1

G. W.[1] (U142101), a 17-year-old boy, was first seen in July 1947, with the complaints of an undescended right testis, sparse pubic and axillary hair and a high-pitched voice. He had never needed to shave, had little libido and had experienced no erections. Previous treatment with 7,000 international units of chorionic gonadotropin had resulted in temporary increase in the size of the right testis and its descent into the lower inguinal canal.

The family history is illustrated in Figure 1. The parents were normal. The patient was the fifth of 6 children. An older brother was married and had children. A married sister, who had no children, was said to have developed normally. A younger brother subsequently matured normally. Two older brothers (Cases 2 and 3) had complaints similar to those of the patient (Fig. 2).

On physical examination the patient was a slender white boy who appeared younger than 17 and who had disproportionately long arms and legs and a high-pitched voice. He was 67¾ inches tall and measured 35½ inches from soles to pubis. His span was 69½ inches. He weighed 130 pounds. Facial, axillary and chest hair were lacking; pubic hair was sparse and horizontally delimited. The penis measured 5 cm. in length and 5 cm. in circumference. The prostate gland was tiny. The left testis was in the scrotum and measured 1½ by 1 cm. A right indirect inguinal hernia was palpable. The right testis was in the inguinal canal; when brought down subsequently by a two-stage Torek procedure, it measured 1 by ¾ cm.

Received for publication December 20, 1954.

[1] Referred by Dr. L. R. Pedemont of Oakland, California.

Urinary pituitary gonadotropin was absent at 10 mouse uterine units per twenty-four hours (method of Klinefelter, Albright and Griswold (7)). Skeletal age was between 12 and 13 years. The serum phosphorus level was 5.3 mg. per 100 ml. On examination of the seminal fluid on two occasions two years after androgen therapy, there were no spermatozoa.

The patient served as a subject for the assay of several testosterone preparations which proved ineffective in stimulating sexual maturation. The details of this study have been reported elsewhere (8). In May 1948, when finally treated with methyltestosterone linguets, 20 mg. daily, he promptly began to have two erections a day, and in one month some acne and mustache hair had appeared. At the end of the second month of therapy his height was 68½ inches, and his penis measured 8½ cm. in length and 8½ cm. in circumference. Five months later he had grown 2 inches and gained 21 pounds. The same dose was continued, and in seven months he was shaving twice weekly and noted that his shirt collars were tight and his shoe size had increased from 9½ to 11. His height at this time was 70 inches, weight 157 pounds, and his voice had become baritone. Pubic hair was plentiful, axillary hair was normal, and a few hairs had

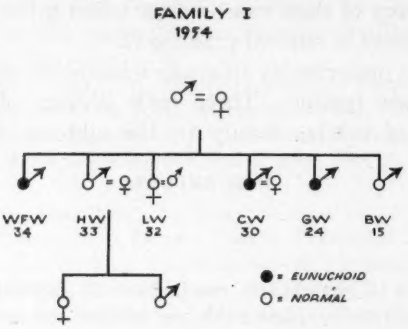

FIGURE 1

appeared about the nipples and on the ears. The penis measured 9½ cm. in length and 9 cm. in circumference. When last seen in 1954, his height was 73 inches (a gain of 5¼ inches) and his weight was 180 pounds (a gain of 50 pounds). He was shaving daily, was muscular and well tanned, had facial acne and a baritone voice. The penis measured 10 cm. in length and 10 cm. in circumference. He had erections three or four times weekly.

Case 2

C. W. (U160776), the brother of G. W. (Case 1, Fig. 1), was 25 years old when first studied in 1948. At the age of 12 he noted that his genitalia were small; they had not developed subsequently. His voice remained high, and he had never shaved. He had always been the tallest boy in his class. At the age of 18 or 19 he noticed that his arms and legs were disproportionately long. Between the ages of 23 and 24, he had grown ½ inch; his height had not increased during the year prior to examination. The right testis had not appeared in the inguinal canal or scrotum. He had occasional erections but no seminal emissions. His libido was normal, and he desired treatment because of contemplated marriage.

On examination the patient was seen to be tall and thin. He had long arms and legs, long, nontapering fingers, and a high voice. His height was 75 inches; 40½ inches were

below the symphysis pubis. His span was 82½ inches, and weight 154 pounds. Pubic hair was thin and horizontally delimited. Facial, auricular and chest hair were lacking. Axillary hair was within normal limits and growth on the legs was abundant. The thyroid gland was barely palpable. The penis was infantile, measuring 4½ cm. in length and 5½ cm. in circumference. The left testis was in the scrotum; it was soft in consistency and measured 1 by 2 cm. The right testis could not be felt. A right indirect inguinal hernia was present. On rectal examination the prostate gland was impalpable.

Urinalyses consistently showed albuminuria and occasional hyaline casts; specific gravity was 1.022. Assay for urinary pituitary gonadotropin was negative at 5 mouse uterine units. The 17-ketosteroid excretion (total neutral fraction) was 10.6 mg. per twenty-four hours. The sella turcica was normal, and the skeletal age was between 16

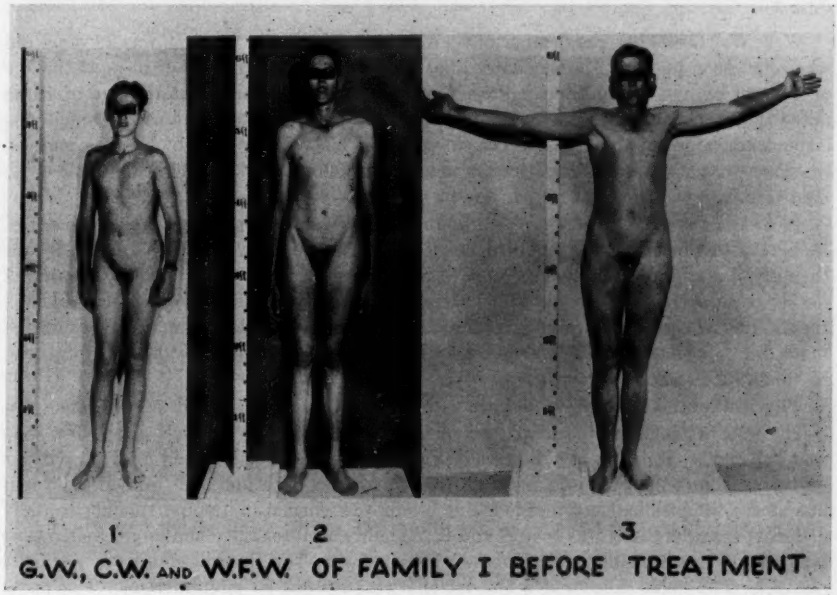

FIGURE 2

and 18 years. The basal metabolic rate was −54 per cent on two occasions, and +10 per cent six weeks after therapy was started. On examination of seminal fluid after the patient had been treated for eighteen months, there were no spermatozoa.

This patient was also the subject of a previous study on the efficacy of various testosterone preparations (8). Administration of unesterified testosterone (Lingusorbs®, Ayerst), 48 mg. a day, resulted in three to four erections a day. After two weeks the patient noted an increased work capacity, and a gain of 4 pounds. The penis measured 8 cm. in length and 7½ cm. in circumference. The right testis had descended into the canal and could be identified at the external ring. After six weeks of treatment his voice had become deeper, and he weighed 165 pounds. In three months he gained 20 pounds and started shaving once a week. Regression took place when another testosterone preparation was used; however, he responded at once when methyltestosterone

linguets were resumed in a dose of 20 mg. daily. Sixteen months after starting treatment his height was $75\frac{5}{8}$ inches, and he weighed 177 pounds. He shaved twice weekly, and reported four to seven erections a day and intercourse once or twice a week. A small amount of tender breast tissue was palpable lateral to the left nipple. The penis measured $9\frac{1}{2}$ cm. in length and $9\frac{1}{2}$ cm. in circumference. The left testis felt normal; the right was in the inguinal canal. He was married about this time.

On his last visit to this Clinic in 1950, the patient's height was $76\frac{3}{8}$ inches (a gain of $1\frac{3}{8}$ inches), and weight 195 pounds (a gain of 41 pounds). He shaved two or three times a week. Temporal recession had started, his voice was deeper, and nipple tenderness had disappeared. The other manifestations of the androgenic effect had been well maintained.

Case 3

W. F. W. (U165714), the brother of G. W. and C. W. (Cases 1 and 2), was 29 years old when first seen in 1949. He had noted that he was different from other boys at the age of 15, and by age 17 considered himself sexually underdeveloped. At the age of 18 he noted that his arms and legs were unduly long. His penis and testes remained small; the latter were migratory. He had never shaved, and his voice was high. He was married when he was 22 years old. Erections occurred three times weekly; there was little or no fluid ejaculate with intercourse. He had no children.

In 1941 he received testosterone propionate injections twice weekly for two months. Following this he had more frequent erections and noted growth of pubic hair. He was then given methyltestosterone linguets (5 mg.), of which he took 6 or 7 daily on and off for six months. In 1943 he purchased some 5-mg. methyltestosterone linguets and took them 6 or 7 times daily for about a month. During this time he had more erections and more fluid ejaculate. In 1949, encouraged by the results obtained by his two brothers, he sought the same treatment.

Physical examination revealed that the patient had eunuchoid proportions. His height was $74\frac{1}{4}$ inches, of which $40\frac{3}{4}$ inches were below the symphysis pubis. His span was $78\frac{1}{4}$ inches, and weight 173 pounds. He appeared prematurely old and showed fine wrinkling about the eyes and mouth. His skin was smooth and well tanned. The hair line at the temples had not receded. Pubic hair was normal in amount but horizontally delineated, axillary hair was sparse, and facial hair was absent. He had large trochanteric fat pads, but no mammary fat pads nor breast tissue. The thyroid gland was normally palpable. The penis measured 5 cm. in length and 6 cm. in circumference. The testes were tiny and soft. The prostate gland was barely palpable. The extremities were long; the fingers did not taper. Dental examination disclosed crowded arches and the absence of the right upper lateral incisor.

A blood count and urinalysis findings were normal. Urinary pituitary gonadotropin was absent at 5 mouse uterine units on two occasions. The 17-ketosteroid excretion (total neutral fraction) was 5.5 mg. per twenty-four hours. The basal metabolic rate was −12 per cent before treatment and −3 per cent after one year of androgen therapy. On examination of seminal fluid, there were no spermatozoa. X-ray films of the skull showed the sella turcica to be somewhat flattened but not definitely abnormal. Epiphyseal lines were present in the head of the humerus, the proximal tibia and fibula and the distal radius and ulna. This was considered indirect evidence of delayed skeletal maturation, since he was then 29 years old.

Treatment was started with a daily dose of 24 mg. of unesterified testosterone (Lingusorbs®, Ayerst) by the buccal route. In one month the patient reported four or

five erections a day, soreness of both nipples, and a weight gain of 4 pounds. His voice was coarser. The testes were no longer migratory; the penis measured 7½ cm. by 8 cm. The dose was reduced to 12 mg. daily. After six months of treatment he felt less tired and was working well. He reported two erections a day and had intercourse every other night. Pubic and axillary hair had increased slightly. The nipple soreness had disappeared. Androgenic effects were maintained by the buccal administration of 10 mg. of methyltestosterone a day. Eight months after starting therapy he had gained 12 pounds and was shaving twice a week. Temporal recession had started, and more hair had appeared on the face, legs and pubic region. After eleven months he had gained 20 pounds, his voice was deeper, the trochanteric fat pads were no longer apparent, and he appeared more muscular. The pubic hair was growing up the linea alba. The penis measured 9 by 9½ cm. When last seen in this Clinic in December 1950, he weighed 197 pounds. He was shaving three times weekly, the pubic and axillary hair was fully developed, and there was more hair on the extremities. The other androgenic effects had been well maintained.

Family II

Case 1

B. McH.[2] (U214389) was first seen in 1953, when she was 17 years of age, because of primary amenorrhea and lack of sexual development. She had been born at full term and weighed 6 pounds, 1 ounce. She was the shortest girl in her class from age 12 to 13 and had remained shorter than average until age 16. She had never menstruated and there had been no development of mammary glands, or of pubic and axillary hair.

In 1952, when 16 years old, she had been treated with thyroid substance and an injection of estrone (1 mg.) and testosterone (10 mg.) every two weeks for five months. During this time she grew 2 or 3 inches, had estrogenic withdrawal bleeding, noted some growth of pubic and axillary hair and slight enlargement of the breasts.

The patient was the second of 3 siblings. The parents and an older sister were normal. A younger brother was also retarded sexually (Fig. 3). A half-brother and half-sister were normal. Other members of the family were said to be normal.

On physical examination the patient was a young woman with no visible breasts, scanty pubic and axillary hair, and no hair on the extremities. She had eunuchoid proportions, and weighed 115 pounds. Her height was 66¼ inches, the lower measurement was 37¼ inches; the span was 69¼ inches. Her arms and legs were long; the fingers did not taper. The carrying angle was normal. Her skin was well tanned, and she had numerous moles. Her neck was not webbed. The thyroid was small. Trochanteric fat pads were well developed. Pelvic examination revealed flat labia majora and small labia minora, no visible clitoris, and an introitus that would not admit one finger. On rectal examination, there was an infantile cervix with a tiny cord-like uterus in the midline. The adnexae were not palpable. Dental examination showed absence of the lateral incisors.

Assays for urinary pituitary gonadotropin were negative at 5 mouse uterine units on two occasions. The 17-ketosteroid excretion was 2.7 and 3.4 mg. per twenty-four hours. The basal metabolic rate was −18 per cent; serum cholesterol level, 201 mg. per 100 ml.; and serum protein-bound iodine level 8.7 micrograms per 100 ml. Insulin tolerance test—fasting blood sugar level, 95 mg. per 100 ml.; after 5.5 units of insulin intravenously, the values were 38, 94, 91, 93 and 94 mg. per 100 ml. On x-ray examination, the sella turcica appeared normal. The skeletal age was approximately 13½ years. A vaginal smear showed atrophic epithelium with a slight hormonal effect. A basal body

[2] Referred by Dr. D. L. Ichelson of Tulare, California.

temperature record before treatment showed wide fluctuations without any pattern.

The patient was then given the monobenzyl ether of stilbestrol (Monozol®) 3 mg. daily. After one month she noted breast tenderness, but the basal body temperature record was unchanged. The breasts were sore on palpation, the nipples and areolae were darker, but there was no appreciable increase in size. There was increased vaginal secretion, and the vaginal mucosa appeared healthy and showed an estrogenic effect.

After three and a half months of uninterrupted treatment, the patient still had slight leukorrhea but no vaginal bleeding had occurred. The breasts were larger; the areolae were deeply pigmented. Pubic hair was slightly increased, the labia were pig-

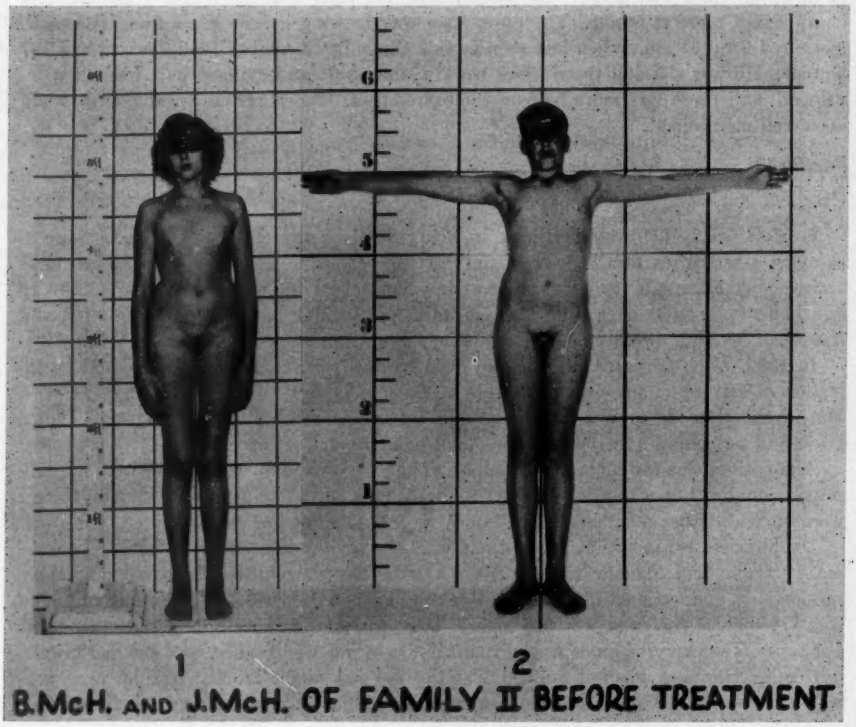

FIGURE 3

mented, and a small clitoris was now apparent. The dose of Monozol® was increased to 6 mg. daily.

After six months the basal body temperature curve continued to be grossly irregular without any pattern. The patient still had not menstruated. Her hips had increased somewhat in size. Her breasts were larger and were moderately tender. At this time (January 25, 1954) she had her first vaginal bleeding, which lasted intermittently for thirteen days despite continued administration of Monozol®. A positive estrogenic effect was observed in the vaginal smear. Assays for urinary pituitary gonadotropin were questionably positive at 5 mouse uterine units and negative at 20 units. The

positive gonadotropin assay was found to be due to estrogen not removed from the urine by kaolin precipitation (9); a modified procedure resulted in removal of the estrogen and in a negative reaction for gonadotropin at 5 mouse uterine units. Since no gonadotropin elaboration was obtained with Monozol® therapy, the patient was given ethinyl estradiol, 0.05 mg., to maintain sexual development and withdrawal bleeding. Withdrawal bleeding occurred on this program, but the patient complained of nausea, and the dose was reduced to 0.02 mg. in cycles of twenty-three days on, and seven days off treatment. The skeletal age had progressed to 17–18 years.

Case 2

J. McH. (U221534), a 16-year-old white boy (brother of B. McH., Case 1), came to this Clinic in 1954 because of failure to develop sexually. His genitalia had always been small, he did not shave, and his voice was high pitched. His libido was normal; he was able to induce a rare erection, but no ejaculation. He was active in sports, but was embarrassed to undress in the gymnasium because of his retardation. He denied sensitivity to cold. He had grown approximately 2 inches and had gained 12 pounds in the previous six months.

On examination, the patient was a well-nourished boy who had eunuchoid proportions. His height was 68¾ inches; lower body measurement, 37½ inches; span, 72 inches; and weight, 150 pounds. His face was not wrinkled. There was no temporal recession. He had fine, fuzzy facial hair, sparse pubic hair and a few axillary hairs. The thyroid gland was normally palpable. The penis measured 4½ cm. in length and 5¼ cm. in circumference. The testes were in the scrotum and were tiny. The prostate was barely palpable. His arms and legs were long; his fingers lacked taper. The carrying angle was normal. Muscle development and strength were normal. Dental examination showed the absence of the lateral incisors.

Urinary pituitary gonadotropin was absent at 5 mouse uterine units. The 17-ketosteroid excretion was 7.1 mg. per twenty-four hours, and after corticotropin stimulation, 7.2 mg. per twenty-four hours. A urinary 17-hydroxycorticoid determination was 6.9 mg., and after corticotropin stimulation, 11.6 mg. per twenty-four hours (method of Reddy *et al.* (10). The basal metabolic rate was −18 per cent; serum cholesterol level, 228 mg. per 100 ml; serum protein-bound iodine level, 6.4 micrograms per 100 ml.; and thyroidal I^{131} uptake, 9.6 per cent (normal, 6 to 23.6 per cent) in three hours. Insulin tolerance test—fasting blood sugar level, 94 mg. per 100 ml; after 6.9 units of insulin intravenously, 24, 63, 82, and 104 mg. per 100 ml. The test was repeated, and the fasting blood sugar level was 115 mg. per 100 ml.; after insulin, 20, 77, 107, 110 and 94 mg. per 100 ml. The serum phosphorus values were 4.4 and 5.7 mg. per 100 ml. The basic phosphatase level was 12 Bodansky units per 100 ml. A lateral x-ray film of the skull showed a normal sella turcica. The skeletal age was 13 to 14 years.

The patient was first given a course of 5,000 units of chorionic gonadotropin (APL, Ayerst), three times weekly. After the third injection he complained of priapism, and by the end of four weeks his voice began to crack. Facial hair and pubic, axillary and extremity hair had increased slightly. There was no recession of the temporal hair line, but acne had appeared on his forehead. The penis measured 6 cm. in length and 8 cm. in circumference; the testes felt slightly larger. His voice was definitely lower in pitch. Buccal administration of methyltestosterone, 5 mg. a day, during the next three months resulted in continued genital development (penis, 8½ cm. long and 7½ cm. in circumference). He had his first seminal emission, and gained 5 pounds in weight and 1½ inches in height. He continued to grow and gain weight, but since genital development did not progress, the dose of methyltestosterone was increased to 10 mg. a day buccally. He

then had three or four erections a day. Axillary and pubic hair became more plentiful, and genital development was maintained.

Examination of the patient for color blindness and anosmia, which may be associated with familial eunuchoidism (6), showed no defect of color vision or sense of smell.

DISCUSSION

Familial hypogonadotropic hypogonadism may appear in association with other congenital defects, or as a selective pituitary failure. Hurxthal (1) described 3 brothers with hypogonadotropic eunuchoidism. They had eunuchoid proportions, and skeletal age was retarded, but there was no evidence of other pituitary deficiency. An assay for urinary gonadotropin had negative results in one of the brothers. Two first cousins with hypopituitarism were reported by Hubble (11). Only one, however, was eunuchoid; the other was a pituitary dwarf. Simpson's patients (2) may also have had hypogonadotropic eunuchoidism, but insufficient data were available to establish this point at the time of his study. Other reported instances of familial hypogonadism were not of the hypogonadotropic variety (3–5).

Familial hypogonadism on a pituitary basis occurs in conjunction with other defects, as in the Laurence-Moon-Biedl syndrome (12). Some doubt as to the secondary nature of the testicular abnormality has been cast by Francke (13), but the hypogonadism is generally thought to be pituitary in origin. Ryan and McCullagh (5) reported 3 brothers with hypothyroidism and varying degrees of hypogonadism suggestive of pituitary deficiency. An assay for urinary gonadotropin was positive at 13 to 26 mouse units in 2 of the patients and negative at 13 units in the third. The fact that 17-ketosteroid excretion was practically zero in each patient suggests lack of other pituitary functions. Secondary hypogonadism was also one of the manifestations of pituitary dwarfism in 2 siblings studied in this clinic.

Primary hypogonadism of the Klinefelter type also may be familial. The largest group reported was the family studied by Reifenstein (14). Nine of 10 males in this family manifested the Klinefelter syndrome; the hereditary factor was apparently transmitted by females.

Primary hypogonadism associated with multiple congenital defects has also been reported in families. Kallmann *et al.* (6) described 12 members of 3 families who had primary eunuchoidism and other defects. Color blindness and mental retardation were found in the noneunuchoid relatives, but anosmia and synkinesia occurred only in the eunuchoids, and not in all of them. The genetic explanation offered for this syndrome is a chromosomal aberration with breakage and partial translocation of the X chromosome. Two brothers reported by Sohval and Soffer (15) had testicular deficiency, diabetes mellitus, multiple osseous anomalies and

mental deficiency. The testicular defect associated with myotonia dystrophica has also been noted to run in families (16, 17).

The diagnosis of the hypogonadotropic type of hypogonadism, unless there is associated evidence of other pituitary dysfunction, can be established only by laboratory confirmation. The complete absence of pubic and axillary hair and truly infantile genitalia suggest a pituitary origin. Urinary gonadotropins are absent. Histologic examination of testicular tissue shows immature tubules, absence of Leydig cells, and no spermatogenesis. Although testicular biopsy is helpful, the findings are not always diagnostic. McCullagh *et al.* (18), in a study of testicular structure and function in 20 cases of organic pituitary disease, concluded there were no histologic characteristics to differentiate primary from secondary testicular failure. Others (19) believe that biopsy is of great diagnostic value in revealing the precise character of the disorder, and urge its use routinely in all cases of hypogonadism. The response to administration of chorionic gonadotropin, which has also been suggested as a criterion for diagnosis (20), is probably too insensitive for this purpose (21).

The difficulty of diagnosing eunuchoidism in teen-age subjects is exemplified in Family II. Obviously, a delay in onset of puberty beyond the arbitrary "normal" age could easily be mistaken for hypogonadism or for hypopituitarism. The eunuchoid proportions and the normal response to insulin-induced hypoglycemia, plus the normal levels of serum protein-bound iodine, thyroidal I^{131} uptake, and 17-ketosteroid excretion in the case of the boy, serve to exclude retarded puberty and "panhypopituitarism."

Therapy with anterior pituitary-like hormones has been shown to be effective in the treatment of secondary eunuchoidism (19–21). Bartter *et al.* (21) found that treatment with chorionic gonadotropin resulted in stimulation of all elements of the testes and in spermatogenesis in 3 of 9 patients. Other observers (20) have reported marked stimulation of the Leydig cells and an increase in the size of the tubules but no increase in spermatogenesis after such therapy. Although this form of replacement therapy is physiologic, the necessity for frequent, prolonged administration by the parenteral route, and the expense and occasional refractoriness due to the production of antihormones (22) render it impractical.

The efficacy of testosterone compounds in the treatment of eunuchoidism has been well established (4, 8, 23–27). Secondary sex characteristics develop in weeks to months and, rarely, spermatogenesis may be produced (28, 29). The ease of administration, the economy and the readily controlled dosage of this form of replacement therapy ("end-organ therapy") are noteworthy advantages. Prolonged administration of testosterone to normal men has been shown to cause changes in the testis

which are completely reversible (30); in fact, the testicular structure after testosterone may be more nearly normal in appearance than in the pretreatment biopsy specimen. In another study (31) no evidence of testicular damage was noted after prolonged intermittent therapy with testosterone. The best form of treatment may be initial stimulation of the testes by gonadotropin, followed by maintenance with methyltestosterone.

In evaluating any form of therapy for presumed hypogonadotropic eunuchoidism, it is important to consider the possibility of spontaneous but retarded sexual development (28). In the classification suggested by Bartter *et al.* (21), there were patients with delayed puberty and "eunuchoidism-with-low-FSH" and some testicular development, who matured during therapy with chorionic gonadotropin and continued to progress after therapy had been stopped. These authors concluded that the effects of treatment can be evaluated only if regression follows discontinuance of therapy.

Of incidental interest in the study of the two families reported here is the confirmation that a low basal metabolic rate occurs in hypogonadism in the absence of hypothyroidism. In Family I, a low basal metabolic rate was found in Case 2 (−54 per cent) and a moderately low rate in Case 3 (−12 per cent). In both instances the rate rose to normal levels after administration of androgens. Moderately low basal metabolic rates were also noted in both cases in Family II, despite normal thyroid function. These observations are in harmony with those of Kenyon *et al.* (32).

SUMMARY

1. Five cases of hypogonadotropic eunuchoidism in two different families are reported. In both families the condition occurred in siblings who had normal brothers and sisters.

2. Growth, thyroid function and adrenocortical function were normal in the 5 cases.

3. Satisfactory genital development was maintained in all 5 cases by "end-organ therapy," *i.e.*, substitution, by means of gonadal rather than gonadotropic hormones.

Acknowledgments

Grateful acknowledgment is made to Ayerst Laboratories, New York, Ciba Pharmaceutical Products, Inc., Summit, New Jersey, and G. D. Searle and Co., Skokie, Illinois, for materials used in this study.

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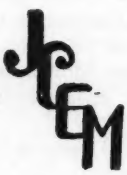

SERUM CORTICOIDS IN LIVER DISEASE*

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IN ORDER to evaluate differences in blood corticoid responses to common stress, factors affecting the disappearance of corticoids from the blood as well as those affecting their appearance must be understood. This study was undertaken to consider the effect of liver disease upon serum corticoids. It was carried out by measuring both the free and the conjugated serum corticoids (acid hydrolyzable) in normal individuals and in patients with liver disease. The measurements were made in subjects studied before and after the ingestion of cortisone, before and after receiving ACTH intravenously and before and after undergoing surgical procedures.

METHODS

The free serum corticoids were measured by the method of Nelson and Samuels (1). The conjugated serum corticoids were measured by an adaptation of this method. The modification, which is essentially an acid hydrolysis of the residue after the Nelson-Samuels extraction, has recently been described from this laboratory (2). This procedure leads to a 50 per cent destruction of pure compound F carried through the hydrolysis and extraction procedure and then measured by the method of Nelson and Samuels. However, over 90 per cent of tetrahydrocortisone is recoverable by this method.

The residue from the Nelson-Samuels extraction of free corticoids is drained from the separatory funnel into a 250-ml. glass-stoppered centrifuge bottle. The separatory funnel is washed out with 10 ml. of concentrated hydrochloric acid (37 per cent) and this acid is drained into the centrifuge bottle. The bottle is then shaken in a mechanical shaker for fifteen minutes. After shaking, the glass stopper is removed from the centrifuge bottle with care and the pressure within the bottle is relieved. Twenty-five ml. of chloroform, freshly distilled as in the original method of Nelson and Samuels, are added to the centrifuge bottle. The material is then shaken once more in the shaking machine and the resultant emulsion is broken by centrifugation. The lower level of chloroform is removed by aspiration with a 30-ml. syringe and a 4-inch 19-gauge needle.

Received for publication November 19, 1954.

* This study was supported in part by a grant from the Public Health Service No. A293 from the National Institute of Arthritis and Metabolic Diseases and by a grant from the Thomas Raymond Evans Foundation.

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The chloroform is placed in an Erlenmeyer flask. This extraction process is repeated twice more and the chloroform extracts pooled in the Erlenmeyer flask. The pooled chloroform extract is then evaporated under an air blast. The dried extract is redissolved in 5 ml. of chloroform and treated in exactly the same manner as the original Nelson-Samuels extracts.

The normal individuals used for controls in this study were laboratory workers, medical students and members of the house staff considered to be in good health, as well as adults and children hospitalized for elective surgery or for the investigation of convulsive disorders or mental retardation. The patients with convulsive disorders had had no convulsions within two weeks of the study carried out in the hospital.

The patients with cirrhosis (except for Patients RH and HE mentioned subsequently) had had the symptoms of this disease for over one year and were jaundiced at the time of the studies. Ascites had occurred in all of them before the tests were made, but was still present in only one, JA. They showed laboratory evidence of marked liver dysfunction as well as the clinical signs, symptoms and history of cirrhosis. The etiology was thought to be infectious hepatitis in 3, and alcoholic and nutritional disorders in the rest. With the exception of 1 patient, TH, all were improving at the time the studies were carried out. Patient TH began to bleed from esophageal varices the day the test was performed and died four days later from uncontrolled bleeding and shock. Patients RY and HE were considered to have early, minimal liver disease. RY had a history of chronic alcoholism and had had delirium tremens on one occasion. He was admitted to the hospital for hypertension and nervousness, and a diagnosis of early cirrhosis was made on the basis of a 2-plus cephalin flocculation, a sulfobromophthalein retention of 12 per cent and the presence of gynecomastia and spider angiomata. He had neither clinical nor laboratory signs of liver involvement by the time the corticoid studies were carried out, and he never was jaundiced. Patient HE had had morning vomiting and ankle edema for about two weeks before entry. On admission an enlarged liver was found and tests showed abnormal hepatic function. He was never jaundiced and after two months in the hospital all physical and laboratory abnormalities had disappeared. At this time, the studies reported here were carried out.

Three children with acute hepatitis in the second week of illness and 1 child with homologous serum jaundice were studied, as was 1 adult who had had chronic hepatitis for approximately one year. Two patients, aged 16 and 26, with hepatolenticular degeneration (Wilson's disease) were also studied. Preoperative and postoperative measurements were made on a 2-month-old child with congenital biliary atresia. Exploratory laparotomy on this patient revealed mild to moderate biliary cirrhosis.

Studies were started between 8 and 9:30 A.M., with a very few exceptions among the earliest studies, which were begun in the late morning. Presumably, these few early studies did not differ in any way from the later ones, as the variation in time from 8 A.M. till noon was not associated with a significant drop in the level of blood corticoids in the controls.

Cortisone acetate tablets were administered to the controls and to the patients with liver disease in doses of 60 mg. per square meter of body surface area. ACTH was given in doses of 10 mg. per square meter of body surface area. The ACTH was administered intravenously within a period of one to four minutes. Surgical procedures in 2 patients with liver disease were exploratory laparotomy and biopsy of the liver in the child with biliary atresia, and inguinal herniorrhaphy in an adult with Laennec's cirrhosis.

RESULTS

The reproducibility of the method for conjugated serum corticoids is demonstrated in Table 1. With one exception, the values in duplicate samples of serum run on the same day, or in a few samples run on successive days (being kept frozen in the intervening period), did not vary more than 10 per cent on either side of the mean.

The average serum level of free corticoids in the group of normal controls was 13 micrograms (μg.) per 100 ml. The average serum level of conjugated corticoids by this method in 30 normal adults was 8.5 μg. per 100

TABLE 1. RESULTS OF REPLICATE ANALYSES OF THE SAME SAMPLES OF SERUM

Serum	Estimation							
	1	2	3	4	5	6	7	8
	micrograms/100 ml.							
A	15	12	13	13	13	13	13	13
B	14	14						
C	6	6						
D	0	0						
E	8	6						
F	13	16						
G	10	9	10*					
H	0	0						
I	0	0	0*					
J	22	13	11*					
K	9	8						
L	16	17	17*	14*	14*			
M	17	15						
N	15	15						
O	19	21						
P	0	0						

* Specimens measured 24–96 hours after original measurements.

ml.; the standard error of the mean was ±1.3 μg. The average levels of free and conjugated corticoids in 15 specimens from 14 adult patients with cirrhosis were 10 μg. and 1.7 μg. per 100 ml., respectively. The differences in conjugated serum corticoids between these random pretreatment measurements in patients with liver disease and in normal individuals was highly significant ($P < .01$).

After the ingestion of cortisone acetate by normal adults the rise in conjugated corticoid levels followed the rise in free corticoid levels. The peak was later and the elevation of conjugated corticoids persisted longer

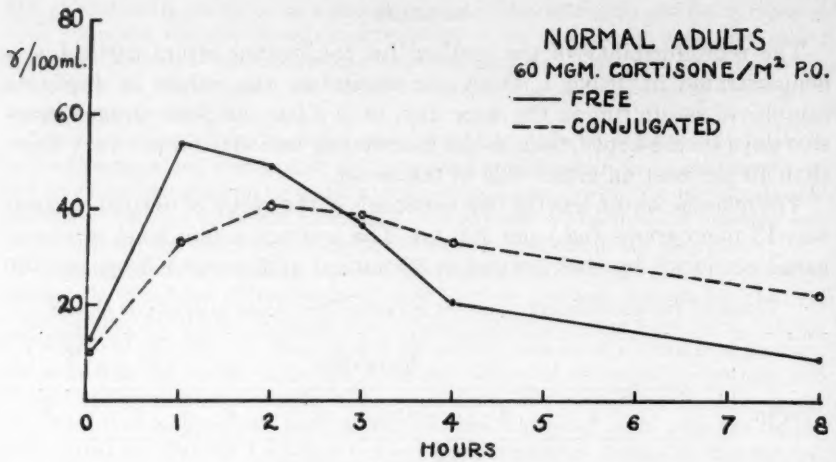

FIG. 1. Serum levels of free and conjugated corticoid in 16 normal individuals, before and after cortisone ingestion. The corticoid level in micrograms per 100 milliliters of serum is plotted against the time in hours after the ingestion of cortisone. The free corticoid level is represented by the dots and solid line, and the conjugated corticoid level by the open circles and broken line.

TABLE 2. SERUM LEVELS OF FREE AND CONJUGATED CORTICOIDS BEFORE AND AFTER THE INGESTION OF CORTISONE ACETATE IN 8 PATIENTS WITH CIRRHOSIS

Cortisone tolerance (60 mg./M² orally)

Cirrhosis patients	Corticoids (μg./100 ml.)									
	Control		1 Hour		3 Hours		4 Hours		8 Hours	
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
RY	16	2	48	17	25	21				
GL	17	1	68	1	60	2				
PI	2	0	90	3	53	4				
DI	3	2	14	0	80	20				
WA	0	0	25	3			34	5		
BR	20	0	103	7					9	6
MA	0	7	85	25					0	2
TH	0	0	0	0					0	3
MEAN	7	1.5	47	7	55	12	34	5	3	4
SEM	±2.8	±0.8	±11.8	±3.1	±9.8	±4.4			±2.5	±1
Diff. from norms	P <.1	P<.01	P >.1	P<.01	P <.01	P<.01			P<.1	P<.1

than that of free corticoid. The two levels approximated each other sometime between two and three hours after the ingestion of cortisone. Table 2 shows the results obtained in studies of 8 patients with cirrhosis who received cortisone acetate by mouth. Figure 1 shows the curves of the average levels of free and conjugated corticoids before and after the ingestion of 60 mg. of cortisone acetate per square meter of body surface area in 16 normal adults. Figure 2 shows a comparison of these normal

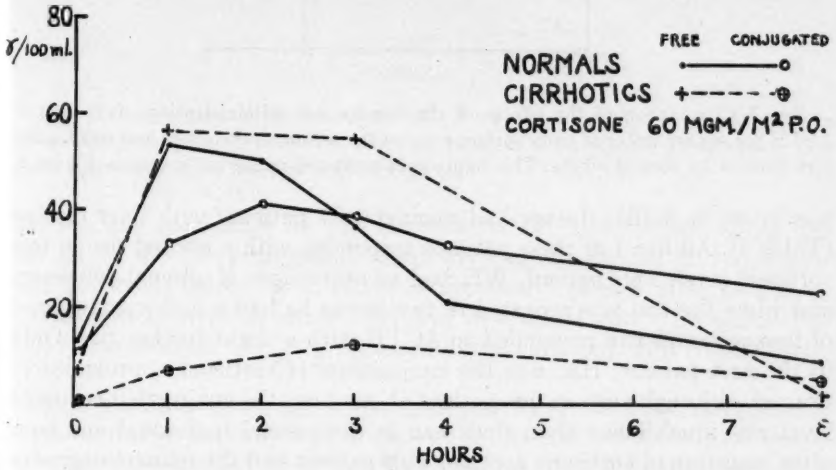

FIG. 2. Comparison of mean serum levels of free and conjugated corticoids in 16 normal adults (Fig. 1) with those in 8 patients with cirrhosis, before and after the ingestion of cortisone acetate. The levels of free and conjugated corticoids in micrograms per 100 milliliters of serum are plotted against time in hours after the ingestion of cortisone. The results in the normal subjects are represented by the solid lines; the free corticoid by solid dots and conjugated corticoid by the open circles. The results in the cirrhotic patients are represented by dotted lines; the free corticoid by crosses and the conjugated corticoid by crossed circles.

curves with the curves of the average levels in the 8 cirrhotic patients who received the same dosage of cortisone acetate.

Table 3 shows the results of similar studies made in 3 children with acute hepatitis, 1 adult with chronic hepatitis and 1 patient with hepatolenticular degeneration. One of these patients received a larger dose of cortisone than any of the patients in the other parts of this study. These patients, too, apparently conjugated administered cortisone less well than the normal individual and maintained a higher level of free corticoid after a given dose of cortisone than did the normal individual.

When ACTH was given intravenously to control subjects there was a prompt rise in both free and conjugated corticoid levels (Fig. 3). ACTH

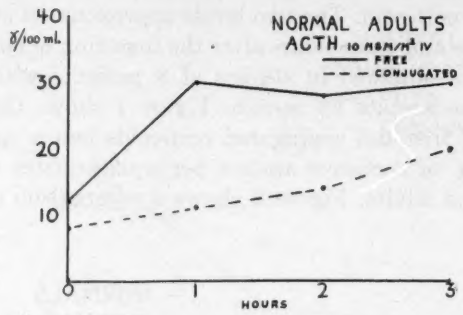

FIG. 3. Comparison of the effects of the intravenous administration of 10 mg. of ACTH per square meter of body surface area on the serum levels of free and conjugated corticoids in 18 normal adults. This figure is constructed in the same way as Figure 1.

was given in similar dosage and manner to 7 patients with liver disease (Table 4). All but 1 of these patients responded with a normal rise in free corticoid level. This patient, WE, had no other signs of adrenal deficiency and when the test was repeated in two weeks he had a high control level of free corticoid and responded to ACTH with a slight further rise. Only in the first patient, HE, was the conjugation of corticoid approximately normal, although even in this patient at one hour the conjugated corticoid level was much lower than that seen in the normal individual one hour after ingestion of cortisone acetate. This patient had the mildest degree of liver disease.

Figure 4 shows a comparison of the average curves after ACTH in 18 normal adults with those in the 7 patients with liver disease.

ACTH was given in similar fashion to a 6-year-old boy with homologous serum jaundice. His free corticoid level rose from 18 μg. to 28 μg. per

TABLE 3. SERUM LEVELS OF FREE AND CONJUGATED CORTICOIDS BEFORE AND AFTER THE INGESTION OF CORTISONE ACETATE IN 4 PATIENTS WITH HEPATITIS AND 1 PATIENT WITH HEPATOLENTICULAR DEGENERATION

Disease	Dose of cortisone (mg./M²)	Corticoids (μg./100 ml.)											
		Control		1 Hour		2 Hours		3 Hours		4 Hours		8 Hours	
		Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
Ac. hep.	60	63	4	156	10			61	21				
Ac. hep.	60	20	6	65	3					67	20		
Ac. hep.	128	36	16			177	11					63	30
Chr. hep.	60	27	10	17	0			65	15				
Mean		*37*	*9*	*79*	*4*	*177*	*11*	*63*	*18*	*67*	*20*	*63*	*30*
Wilson's disease	65	4	0	65	16							13	12

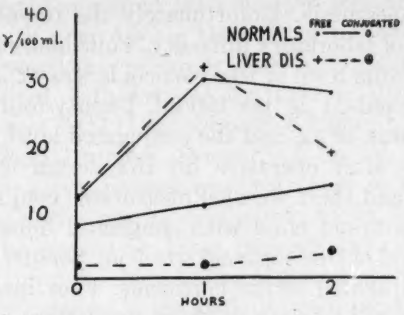

FIG. 4. Comparison of the effects of ACTH on the serum levels of corticoids in normal subjects (Fig. 3) with the effect in patients with hepatic dysfunction. This figure is constructed in the same way as Figure 2.

100 ml. one hour after he had received ACTH and reached 32 μg. per 100 ml. at the two-hour point. There were no measurable acid-hydrolyzable corticoids before, or at either of the hourly periods after ACTH.

The patient with Laennec's cirrhosis who underwent a herniorrhaphy had no measurable free or conjugated serum corticoids two minutes after

TABLE 4. FREE AND CONJUGATED SERUM CORTICOIDS IN 7 PATIENTS WITH LIVER DISEASE, BEFORE AND AFTER RECEIVING ACTH. PATIENT KA HAD WILSON'S DISEASE, THE OTHERS HAD CIRRHOSIS. TWO STUDIES WERE CARRIED OUT ON PATIENT WE, TWO WEEKS APART

ACTH (10 mg./M² i. v.)						
Cirrhosis patients	Corticoids (μg./100 ml.)					
	Control		1 Hour		2 Hours	
	Free	Conj.	Free	Conj.	Free	Conj.
HE	4	11	34	1	21	17
SE	15	3	42	2	34	8
KA	6	0	33	8	10	4
HA	6	0	27	1	2	0
VI	3	0	22	0	29	0
WE 1	8	0	8	0	6	0
WE 2	39	0	48	1	0	0
JA	16	0	39	0	50	0
MEAN	12	2	32	2	19	4
SEM	±3.9	±1.3	±4.2	±.9	±5.2	±2.1
Diff. from norms	P>.1	P<.05	P>.1	P<.01	P>.1	P<.01

the beginning of anesthesia. Unfortunately the pre-anesthetic level was unreliable because of laboratory difficulty. Four hours after the beginning of operation, the serum level of free corticoids was 42 μg. per 100 ml. and of conjugated corticoids, 1 μg. per 100 ml. Twenty-four hours after operation the free level was 40 μg. and the conjugated level, 10 μg. per 100 ml. Seventy-two hours after operation his free serum corticoids measured 22 μg. per 100 ml. and there were no measurable conjugated serum corticoids. In the 2-month-old child with congenital biliary atresia the preoperative serum level of free corticoid was 5 μg. per 100 ml. and there were no measurable conjugated serum corticoids. Four hours after operation the free corticoid level had risen to 58 μg. per 100 ml. and there still were no measurable conjugated serum corticoids. Studies in 18 normal individuals undergoing surgery showed an average free corticoid level of 52 μg. per 100 ml. four hours after operation, and an average conjugated corticoid level of 21 μg. per 100 ml. (2).

The average concentrations of corticoid, both free and conjugated, in 3 patients with acute renal tubular necrosis and anuria and in 2 patients with uremia apparently associated with chronic glomerulonephritis were 11 μg. and 100 μg. per 100 ml., respectively. These were random specimens and were obtained over a wide range of time between 8 a.m. and 4 p.m.

DISCUSSION

In the determination of blood corticoid levels the varied rates of recovery obtained with the method used in this work are analogous to the results obtained with similar methods of hydrolysis of urine. The pitfalls are obvious but the method has the advantages of being easy to perform on large numbers of specimens and of having good reproducibility. For the purposes of this study the reproducibility of the method and the consistency of the response curves were more important than the exact magnitude of conjugated corticoid in any individual.

It is apparent that the patient with advanced liver disease has a defect in the conjugation of exogenous and endogenous corticoid. This apparently is related, albeit crudely, to the degree of liver dysfunction. If the patients with the mildest liver disease and the one patient (TH) who apparently did not absorb the cortisone at the normal rate if he absorbed it at all, are eliminated, the patients with liver disease had a higher peak level of free serum corticoid after a given dose of cortisone by mouth than did the normal subjects. From the two-hour point on, the average level of serum corticoid was higher in all these patients than in the normal individuals so studied. The etiology of the liver disease apparently did not influence the results in this small group.

It is possible that the higher free corticoid levels found in these patients after the ingestion of cortisone were the result of less destruction of the

hormone in the gastro-intestinal tract and/or greater absorption. However, in patients with liver disease the rate of removal of intravenously administered hydrocortisone is slower than in normal persons (4).

Because the patient with hepatic dysfunction disposes of corticoids slowly, a given dose of ACTH should induce higher blood levels of corticoids than in the normal subject. Since this is not so, several possibilities must be considered. First, adrenal responsiveness in these patients may be depressed. Second, the rate of destruction of ACTH or its volume of distribution may be greater in these patients than in normal persons. However, only 1 of our patients had clinical ascites at the time of the study.

The failure of conjugation in the patients with hepatitis seemed similar to that seen in cirrhosis, but the pre-cortisone levels of free corticoid in these patients were high.

There is no evidence at present that conjugated serum corticoid exerts any metabolic effect. The conjugated serum corticoid level rises in the presence of normal free corticoid during renal failure, but elsewhere it reflects the previous level of free corticoid. Since the patient with hepatic dysfunction has a higher level of free corticoid after a given amount of endogenous or exogenous corticoid and maintains this level longer than the normal individual, he should be more affected by a given dose of cortisone than is the normal person. This study does not eliminate the possibility that hepatic metabolism of corticoids may be depressed in other ways than by impairment of conjugation. One would predict this would be so. The limiting factor in the production of conjugated corticoid may not be in the actual process of conjugation but may be a defect in metabolism of corticoids before they can be conjugated. Credence is lent to the latter possibility by the fact that the principal conjugated corticoid in the urine is tetrahydrocortisone (3).

No explanation is immediately evident for the remarkable morning variation in free corticoid levels in Patient WE, or for his failure to respond to ACTH during the first test.

SUMMARY

Patients with advanced liver dysfunction have been shown to conjugate endogenous or exogenous corticoid slowly. The rate of removal of corticoid from the serum is slower than in the normal person. When a patient with hepatic dysfunction is given ACTH, the resulting level of free corticoid in serum is equivalent to that reached in the normal subject given a similar dose of ACTH.

Acknowledgments

The authors wish to acknowledge their gratitude to Dr. T. S. Danowski for permission to study one of his patients, and to Dr. J. R. Woods, Jr. for his help in carrying out the

studies on his patients. They further want to acknowledge their debt to Dr. Robert Schwartz, Chief, Department of Medicine, Veterans Hospital, Aspinwall, for permission to study the patients on his service and for his advice and encouragement. The help of Dr. Richard M. Cyert in preparation of the manuscript is also gratefully acknowledged.

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THE EFFECTS OF SERIOUS ILLNESS AND SURGICAL STRESS ON THE CIRCULATING THYROID HORMONE*

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NOT infrequently it is necessary to evaluate the functional state of the thyroid of critically ill patients. Appraisal on clinical grounds alone may be difficult in patients suffering from associated diseases, and since the basal metabolic rate may be unreliable under these circumstances, appraisal of thyroid function by determination of the serum precipitable iodine (SPI) concentration is frequently employed. Consequently, it is necessary to know with certainty whether or not serious illness, acute or chronic, will influence the level of the circulating thyroid hormone in man.

Studies in experimental animals have suggested that environmental stress (such as exposure to cold, violent exercise, injections of formalin) may alter thyroid function by depressing the rate of thyroidal uptake of radioiodine (1, 2, 3) and may also increase the rate of peripheral utilization of thyroid hormone (4). These combined effects should have a tendency to lower the concentration of circulating thyroid hormone. Furthermore, the adrenocortical hormones, which are known to increase during stress, have been reported to depress the iodine-accumulating function of the thyroid in animals without altering the rate of release of hormone from the gland (5, 6). In man, however, Perry and Gemmell (7) detected no change in the SPI in 11 patients who were subjected to elective, relatively minor surgical operations, whereas Hetzel and associates (8) reported wide fluctuations of the SPI in persons subjected to psychologic stress. Because of these conflicting results, we studied the effects of minor and major surgical procedures on the SPI within hours and days after the operation. Also, the SPI level and the clinical and nutritional status were evaluated in 32 patients who were critically ill from various disorders. In order to test whether cortisone alters the rate of peripheral utilization of thyroid

Received for publication November 20, 1954.

* Supported in part by a grant from the United States Public Health Service and a grant from the Milwaukee Chapter of the American Cancer Society.

hormone, cortisone was administered in large amounts to patients with myxedema (primary, or secondary to anterior pituitary insufficiency) who were maintained on continuous doses of desiccated thyroid. Under these circumstances the possible direct effect of cortisone on the thyroid gland itself was eliminated.

The serum precipitable iodine (SPI) concentration was determined by the digestion-distillation method as modified by Danowski and associates (9). The levels of serum proteins were determined by a modified biuret technique (10). The characteristics of the patients included in these studies are outlined in the following sections.

RESULTS

The effect of surgery on the SPI

The serum protein-bound iodine level was followed in 24 patients who were operated upon for various conditions. The types of operation per-

TABLE 1. TYPES OF SURGICAL PROCEDURE PERFORMED IN THE 24 PATIENTS WHOSE SPI LEVELS WERE FOLLOWED PREOPERATIVELY AND POSTOPERATIVELY

Surgical procedure performed	No. of patients
Saphenous ligation, bilateral	2
Hysterectomy, abdominal	5
Herniorrhaphy, inguinal	4
Sympathectomy, unilateral, lumbar	3
Arthrodesis, hip	2
Mastectomy, radical	2
Amputation, leg	1
Gastric resection, subtotal	1
Laparotomy, ovarian cyst	1
Common bile-duct exploration, carcinoma pancreas	1*
Thoracotomy, carcinoma lung	1*
Colostomy, carcinoma colon	1*

* Expired postoperatively (*see* Figure 1).

formed are outlined in Table 1. It may be noted in Figure 1 that the SPI fluctuated in no consistent direction in 16 of 19 patients followed for five days or more after the surgical procedure; in fact, it remained remarkably constant. In Figure 2 it is shown that the SPI levels in 5 patients remained constant when determined several hours after surgery. Thus an acute surgical procedure, even of considerable magnitude, does not effect the SPI in man. However, it is of considerable interest that the SPI level progressively fell to abnormally low levels in 3 patients who expired five, six

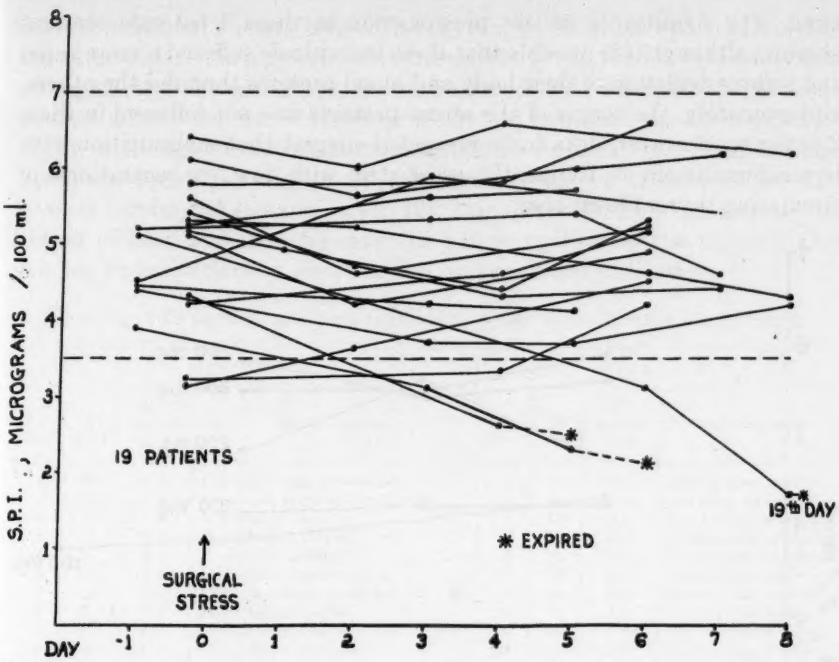

FIG. 1. The course of the SPI for several days following the stress of surgery.

and nineteen days postoperatively (Fig. 1). The causes of their deaths are given in Table 1. None of the other patients who were operated upon ex-

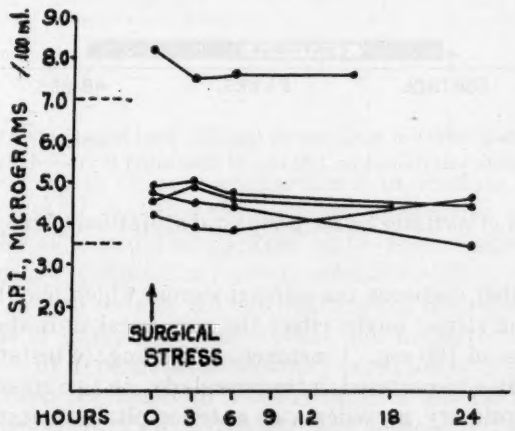

FIG. 2. The course of the SPI for several hours following the stress of surgery.

pired. The significance of this phenomenon in these 3 patients remains obscure, although it is possible that these individuals suffered a more rapid and serious depletion of their body and blood proteins than did the others. Unfortunately, the course of the serum proteins was not followed in these 3 patients. However, data to be presented suggest that malnutrition with hypoalbuminemia is frequently associated with low concentrations of circulating thyroid hormone.

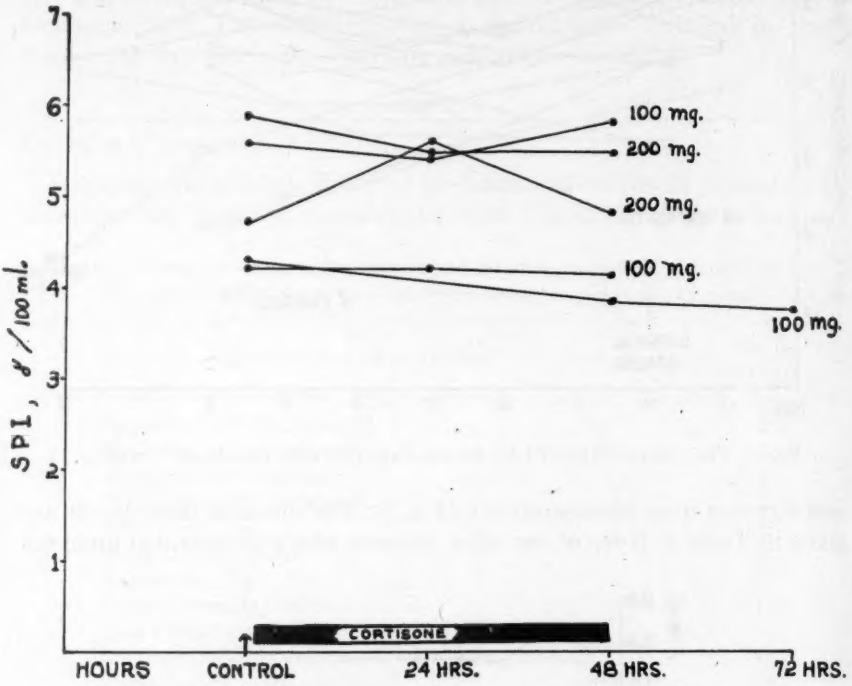

FIG. 3. The lack of effect of cortisone on the SPI level in patients with hypothyroidism maintained on 120 mg. of desiccated thyroid daily.

The acute action of cortisone on the peripheral utilization of exogenous thyroid hormone

To test whether cortisone (an adrenal steroid which may be elaborated in excess during stress) might effect the peripheral utilization of thyroid hormone, a dose of 100 mg. (1 instance) or 200 mg. (4 instances) of cortisone was acutely administered, intramuscularly, on two successive days to patients with primary myxedema or anterior pituitary insufficiency. All had been maintained on 120 mg. of desiccated thyroid daily and the SPI

was at a normal control level. The thyroid medication was continued in the same dosage during cortisone administration. In Figure 3 it is seen that no significant change in the concentration of SPI occurred; if cortisone acutely affects the rate of disposal of thyroid hormone, the effect must be slight. Although prolonged administration of cortisone or ACTH in large dosage to previously euthyroid individuals is known to depress the SPI level in occasional instances (11, 12), this effect seems to be due to the action of cortisone in depressing the iodine uptake by the thyroid. Our studies do not suggest a direct action on peripheral utilization.

TABLE 2. SPI LEVELS, AGE, AND DIAGNOSIS IN THE 32 CRITICALLY ILL PATIENTS

20 Chronically ill				*12 Acutely ill*			
Patient	Age (yrs.)	SPI (μg./100 ml.)	Major diagnosis	Patient	Age (yrs.)	SPI (μg./100 ml.)	Major diagnosis
1	80	1.2	Carcinomatosis, abdominal*	1	71	3.1	Cerebrovasc. accident
2	60	1.3	Carcinoma esophagus, debility*	2	56	3.6	Coronary occlusion*
3	76	1.5	Hip fracture, debility	3	83	3.6	Cerebrovasc. accident*
4	58	1.8	Heart failure, debility	4	58	3.7	Cerebrovasc. accident
5	73	2.3	Pneumonia, debility*	5	71	3.8	Cerebrovasc. accident*
6	65	2.4	Heart failure, debility*	6	60	3.8	Coronary occlusion
7	69	2.6	Carcinoma scalp, metastasis	7	66	4.8	Cerebrovasc. accident
8	81	2.6	Heart failure, severe*	8	61	5.1	Cerebrovasc. accident*
9	70	2.6	Hemachromatosis, debility*	9	68	5.2	Cerebrovasc. accident*
10	61	2.7	Heart failure, diabetes	10	80	5.2	Cerebrovasc. accident
11	59	3.0	Gangrene foot, heart failure	11	70	5.5	Coronary occlusion
12	67	3.0	Carcinoma lung, debility*	12	72	5.9	Cerebrovasc. accident
13	80	3.2	Uremia, debility*				
14	82	3.2	Pneumonia, debility*				
15	76	3.8	Carcinoma stomach*				
16	46	3.8	Hypertension and uremia				
17	71	4.0	Heart failure, pneumonia, debility				
18	66	4.7	Heart failure, cerebrovasc. accident*				
19	68	5.5	Carcinoma esophagus*				
20	59	6.4	Carcinoma cervix, metastasis*				
Mean	67	3.0		Mean	68	4.4	

* Expired.

The SPI level of critically ill patients

It is not possible to define a critical illness in absolute terms. We can merely state that at the time these studies were made, the chances for survival of the patient did not appear to be good. Patients with acute illnesses (coronary occlusion or cerebrovascular accident) were studied as well as patients with serious, chronic, debilitating disorders (for example, carcinomatosis or intractable heart failure and malnutrition). Generally, those who were ill from an acute coronary occlusion or a cerebrovascular accident had been previously in a better state of general health and nutrition than the others. Twelve patients were acutely ill, 20 patients more chronically ill (Table 2), and all were "critically" ill on clinical grounds.

When the initial SPI levels of all 32 patients are compared with our normal range for 117 control subjects (Fig. 4) it is clear that many abnormally low values were observed. The distribution curve is shifted to the lower range and the mean for the group is 3.5 micrograms per 100 ml., compared with our normal mean of 5.1 micrograms.

The lowest values were noted in the group who were chronically rather than acutely ill; in fact, most of the values for SPI in the acutely ill patients

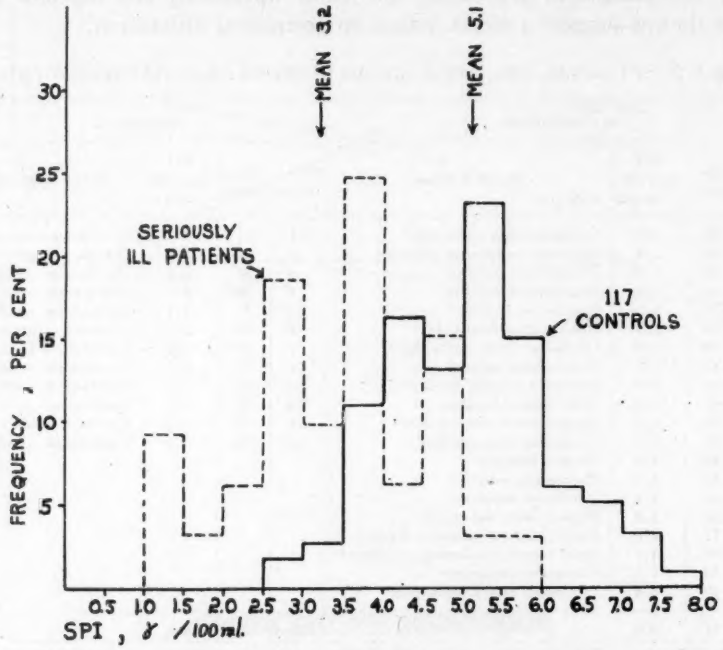

FIG. 4. A comparison of the distribution of SPI levels in a group of normal subjects (solid lines) with the distribution in 32 seriously ill patients (dotted lines). The lowest values were found in the chronically ill group (*see* Table 2).

(cerebrovascular accident or coronary occlusion) were within the normal range. Values below 3.5 micrograms per 100 ml. were noted in 14 of the 20 chronically ill patients and in only 1 of the 12 acutely ill (Table 2). Thus, debility and perhaps chronicity of the illnesses studied, appeared to be the major factor which conditioned the finding of a low SPI level. Whether or not the patient survived the illness did not seem to be important in this regard. In some of these critically ill patients the SPI concentration was followed for several days before death. When abnormally low values were observed, the levels tended to remain low for a

considerable period before death (Fig. 5). This finding further suggests that the low level of circulating hormone is not merely a brief agonal phenomenon but is related in some way to the nature of the illness itself.

Relation of SPI and serum albumin levels

Aside from the critical nature of the illness, the most constant and prominent clinical feature of the chronically ill patient was his generally poor nutritional state. Consequently, we endeavored to determine whether the concentration of circulating hormone was related to the level of the

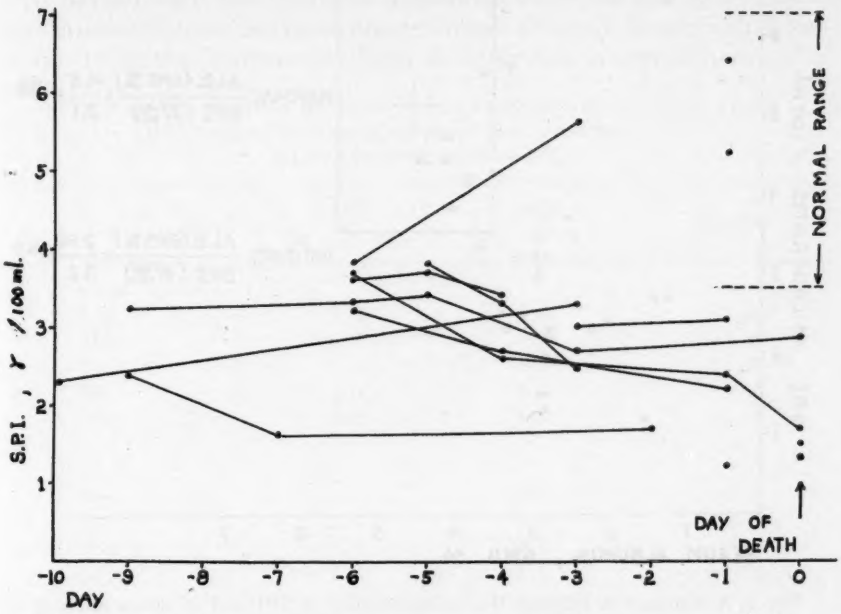

FIG. 5. The course of the SPI prior to death in a group of seriously and chronically ill patients.

serum albumin. There was a significant correlation between these two variables (Fig. 6). In both acutely and chronically ill patients, the ratio of mean serum albumin (in grams per cent) to mean SPI (in micrograms per 100 ml.) was 0.93. The ratio for our normal individuals was 0.88. Although there was a moderate degree of scatter of the individual points, the relationship was linear. None of our patients suffered from significant renal disease, but a similar correlation was noted by Recant and Riggs (13) in patients suffering from nephrosis.

It is generally accepted that the SPI level usually accurately reflects the concentration of circulating thyroid hormone (thyroxine), because the

latter attaches itself to the serum proteins. However, it was conceivable to us, that in these ill patients the SPI concentration might not be accurately measuring the true quantity of circulating hormone if the thyroxine-protein complex did not exist or had become more dissoluble. To test this possibility butanol-extractable iodine concentrations were determined and compared with the SPI level in a few instances (Table 3). In those patients

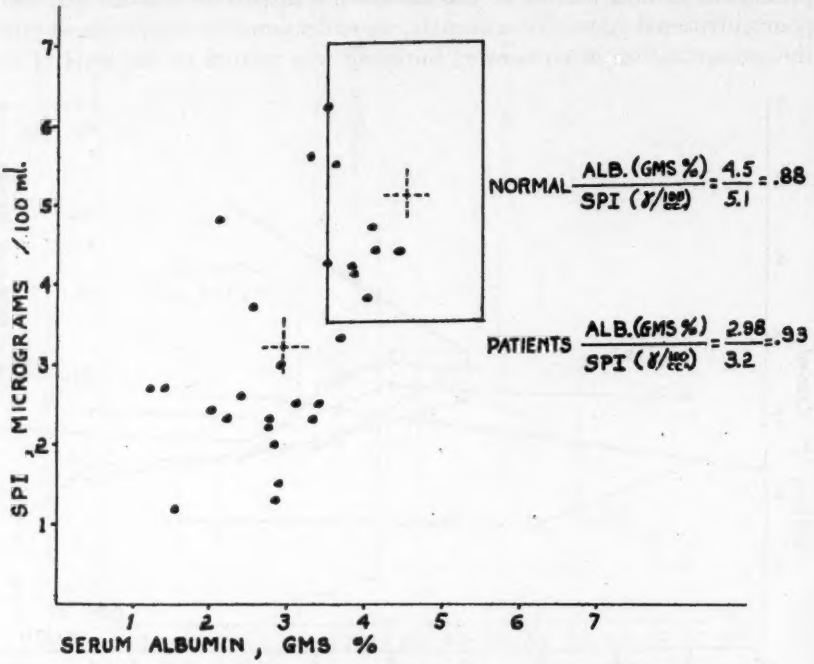

FIG. 6. A comparison between the concentrations of SPI and of serum albumin in 28 of the seriously ill patients. The approximate normal range is depicted as a rectangle. The crossed dotted lines represent the means for SPI and albumin of the patients and normal individuals.

whose SPI concentration was low, the butanol-extractable iodine (thyroxine-iodine) level was also low. This supports the idea that the thyroxine content of serum is, in fact, low under these circumstances. That increased amounts of triiodothyronine might be present remains a possibility, but triiodothyronine is also soluble in butanol (14).

COMMENT

Our data clearly indicate that the stress of a major surgical procedure does not alter the serum concentration of the iodine bound to protein.

Further, even an acute, though serious nonsurgical illness (for example, a coronary occlusion) is not usually associated with abnormal concentration of SPI. Thus, although acute stress may alter the ability of the thyroid to trap iodine, this effect is not reflected in altered concentrations of circulating hormone. It is evident that considerable confidence can be placed in the use of the SPI as a measure of thyroidal activity, even in the acutely ill patient. As a diagnostic technique for assay of thyroid function in the ill patient, the SPI has an advantage over the basal metabolic rate and possibly also some advantage over tracer techniques with radioiodine.

It has not been possible for us directly to study the rate of peripheral utilization of thyroid hormone during stress. Although Bondy and Hagewood (4) felt that cortisone inhibited the utilization of thyroid hormone in

TABLE 3. A COMPARISON BETWEEN SPI AND BUTANOL-EXTRACTABLE IODINE (BEI) LEVELS IN NORMAL SUBJECTS AND IN PATIENTS WITH A LOW CONCENTRATION OF SPI

	SPI (μg./100 ml.)	BEI (μg./100 ml.)	Difference
Normal subjects			
Lyp.	5.5	3.6	−1.9
Mck.	5.7	3.9	−1.8
Vel.	5.5	4.1	−1.4
Blu.	4.0	2.6	−1.4
Mey.	5.2	4.0	−1.2
Patients			
And.	3.3	2.2	−1.1
Far.	2.4	2.0	−0.4
Loo.	1.8	1.7	−0.1
Alb.	1.2	1.9	+0.7

rats, we found no evidence that cortisone in any way acutely influenced its utilization in man. The fact that the SPI level is not influenced by uncomplicated surgical stress does not preclude the possibility that the circulating hormone may be more rapidly altered or utilized during acute stress, since these alterations may be quantitatively very small, or the hormonal output by the thyroid may quickly and concomitantly increase. Shipley and MacIntyre (15) felt that they demonstrated an increase in thyroidal secretion of hormonal radioiodine during surgical stress. However, the effect was noted in only 3 of 9 patients. Whatever may occur, the adjustments to stress in man appear to be sufficiently rapid and quantitatively adequate to maintain constancy of the SPI level in most circumstances of traumatic stress.

On the other hand, in chronic, serious illnesses of diverse nature, low concentrations of SPI are frequently encountered. Malnutrition was the common clinical feature of most of our cases and the SPI level was correlated with the level of the serum albumin. As in patients with nephrosis (13), none of our patients exhibited signs of myxedema in spite of the low concentration of SPI. This phenomenon can not be due to the presence of normal amounts of thyroxine unbound to protein, because the concentrations of butanol-extractable iodine were also low. A true lowering of normal circulating thyroid hormone is therefore strongly suggested. The fact that the administration of albumin to patients with nephrosis does not raise the SPI level (16), also supports this idea. However, our studies do not reveal whether the observed low concentrations of hormone were due to increased peripheral utilization, or to decreased (actual or relative to rate of utilization) formation and release of hormone by the thyroid, or to both factors. The fact that we (17) and others (16) have usually observed normal concentrations of SPI in patients with anorexia nervosa suggests that mere deprivation of food, with its ultimate malnutrition, is usually not sufficient to cause a low SPI level. It appears that an active, associated illness is necessary. That the phenomenon can develop rather rapidly is suggested by the fall in SPI concentration which was observed in the 3 patients who expired in the postoperative period.

From these observations, it is evident that when the SPI concentration is employed diagnostically, caution must be exercised in attributing the finding of a low level of SPI to true myxedema, especially if the patient is chronically ill and suffering from malnutrition and hypoalbuminemia.

SUMMARY

1. Uncomplicated surgical stress, even of considerable magnitude, is not associated with unusual fluctuations of the serum precipitable iodine (SPI) level. Other acute, medical illnesses are not usually associated with abnormal concentrations of SPI.

2. Since the acute administration of 100 or 200 mg. of cortisone did not alter the SPI level in thyroid-deficient individuals during maintenance on exogenous thyroid, it appears that cortisone does not appreciably alter or directly effect the peripheral utilization of thyroid hormone in man.

3. Serious chronic illnesses, especially if accompanied by malnutrition and hypoalbuminemia, are frequently associated with abnormally low values of SPI. In our series of cases there was a close correlation between the level of SPI and that of the serum albumin. The fact that the concentration of butanol-extractable iodine was also low suggests that a true lowering of circulating thyroid hormone took place.

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A CASE OF PSEUDO-PSEUDOHYPOPARATHYROIDISM

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IN 1942, Albright and his collaborators described 3 cases characterized by: 1) clinical signs of tetany associated with hypocalcemia and hyperphosphatemia; 2) a characteristic physical habitus, and bone abnor-

FIG. 1. Patient G. U. Note roundish face, curvature of forearms, left genu valgum.

malities involving mainly the metacarpals and metatarsals; 3) a propenpensity on the part of the subcutaneous tissue to form bone, and usually

Received for publication November 29, 1954.

calcification of the basal ganglia of the brain; and 4) failure of injected parathyroid extract to induce phosphorus diuresis. Albright attributed the syndrome to a failure of the organism to respond properly to its own parathyroid hormone (1).

The syndrome was further studied by Albright's group in 1950 (2) and it was speculatively concluded at the time that the disturbance at the end-organ for parathyroid function, the dyschondroplasia and the metastatic calcifications were three independent defects due to a genetic disorder. It was therefore concluded that, clinically, any one of the defects could be present without the other. This proved to be true in a case presented by Albright and collaborators in 1952 (3). The patient was a 20-

2 3

FIG. 2. Patient's right hand. All the fingers of approximately equal length.
FIG. 3. Patient's left hand. Index finger shorter than the other fingers.

year-old woman, with typical appearance, short metacarpals I, IV and V, absence of calcification of the basal ganglia, lack of response to injected parathyroid extract, but normal serum calcium and inorganic phosphorus levels. In characteristic fashion, Albright coined the new syndrome " 'a-hypocalcemic-pseudohypoparathyroidism', or, more correctly, an 'a-hyperphosphatemic-pseudohypoparathyroidism', or less awkwardly, 'pseudo-pseudo-hypoparathyroidism.' "

We wish to report another case, in some respects similar to that of Albright *et al.*

CASE REPORT

G.U., a 40-year-old white male was admitted to the Vargas Hospital on July 2, 1950, complaining of acute right-flank pain, radiating to the groin on the same side, of two days' duration. The pain had begun suddenly forty-eight hours before, had disappeared

following injections by the local physician, but had returned the day before admission

The patient was born in Caracas and had lived there all his life. His development was normal until the age of 4 years. At this time there began to appear various bone deformities which increased gradually until he was 18 years old, when he had reached his present status. He had never had paresthesias, muscular twitchings, convulsions or tetany. At the age of 34, bilateral cataracts were surgically removed. There was no familial history of endocrine disorders. He had 2 brothers and 2 sisters, all in good health; they were said not to resemble him. There were 2 sons and a daughter, normal in every way. The patient had had a chancre and gonorrhea at the age of 24, and he had been treated with bismuth and arsenicals for one year.

Physical examination: The patient's temperature was 36.4° C., pulse 86, respirations 14, blood pressure 130/90, height 154 cm., and weight 54.5 Kg. He was of short stature, with a roundish face (Fig. 1). The hair and nails were of normal texture. The crystalline bodies of the eyes had been surgically removed, but the fundi were normal; the sclerae had a bluish tinge. The thyroid was not palpable. The heart and lungs were clinically normal. There was slight tenderness in the left flank, which disappeared two days after admission. The liver was palpable two fingerbreadths below the costal margin. The external genitalia were normal. Deep reflexes were somewhat hyperactive; Chvostek and Trousseau signs were absent. The forearms were curved, with the convexity on the radial side. There was left genu valgum. In the right hand, the fingers were of approximately equal length; in both hands, they were short and thick (Figs. 2 and 3).

X-ray examination: There were numerous exostoses, particularly at the origins and insertions of the skeletal muscles (Figs. 4 and 5). Metacarpals III, IV, and V of the right hand appeared shorter than normal (Fig. 6). Bone density was approximately normal. The feet were not examined by x-rays. It was not possible to demonstrate a renal calculus, either with a plain roentgenogram of the abdomen or after retrograde pyelograms. A skull roentgenogram was normal; there were no abnormal calcifications and the sella turcica was of normal size. There was no hyperostosis.

Laboratory studies: On admission the urine was turbid and acid, with a density of 1015, proteins 2+, and numerous leucocytes; later this abnormality disappeared. At no time were there red cells, casts or hemoglobin. Urine culture was not carried out. The blood urea nitrogen level was 8 mg./100 ml., and the fasting blood sugar level, 86 mg./100 ml. Blood sedimentation rate (Westergren) was 30 mm./hour. Wassermann, Kahn and Kline reactions were negative. Hemoglobin: 15.8 Gm./100 ml. Red blood cells: 4,980,000 /cu. mm. White blood cells: 8.000/cu. mm.—neutrophils, 61%; lymphocytes, 38%; monocytes, 1%. Feces contained no ova or parasites.

Serum calcium: 11.0, 11.7 and 11.8 mg./100 ml. on three separate determinations. Serum inorganic phosphate: 2.0 and 2.9 mg./100 ml. Alkaline phosphatase: 3 King-Armstrong units. Serum protein: 6.89 Gm./100 ml.

Ellsworth-Howard test: The patient was given a calcium-poor diet for four days. Urine was then collected hourly, from 6 A.M. to 1 P.M., and Parathormone (Lilly), 200 units, was injected intravenously at 9.15 A.M. The results were as follows:

Time	*Urinary volume (ml.)*	*Phosphorus (mg./hr.)*
6–7 A.M.	196	7.1
7–8	114	9.0
8–9	230	11.8
9–10	122	14.6
10–11	250	18.3
11–12 NOON	102	30.0
12–1 P.M.	44	23.2

4

5

FIG. 4. Roentgenogram of the pelvis and femoral epiphyses, showing exostoses at origins and insertions of muscles.

FIG. 5. Roentgenogram of the knees, also showing exostoses.

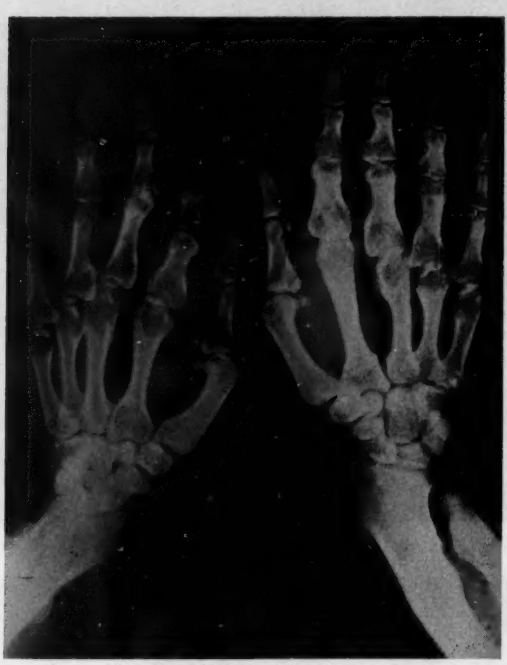

FIG. 6. Roentgenogram of the hands. Right metacarpals III, IV and V, and possibly left metacarpals I and V are shorter than normal.

Unfortunately, it was not possible to perform the test on a normal patient with the same lot of Parathormone. The result of a Sulkowitch test for urinary calcium was similar to that in a normal control.

DISCUSSION

The appearance of the face and of the hands, together with the x-ray finding of short right metacarpals III, IV and V, suggested to us the diagnosis of pseudo-hypoparathyroidism, but we were puzzled by the absence of tetany or convulsions, by the absence of Chvostek and Trousseau signs, and by the presence of a normal level of serum calcium and a rather low level of serum inorganic phosphate.

The case became clearer upon Albright's publication in 1952 (3), and would tend to confirm Albright's idea that the physical characteristics of pseudo-hypoparathyroidism, and possibly the abnormal response to parathyroid extract, may exist without the humoral findings. It is interesting that both in the present case and in Albright's case, there were no subcutaneous or intracranial calcifications. These would seem to be related to the disturbance in calcium metabolism rather than to the disease itself.

It is unfortunate that we could not control the parathyroid preparation used in the present case, but it is interesting that Albright's patient did not respond to the extract, and behaved in this respect as a "true" case of pseudohypoparathyroidism.

SUMMARY

The case is presented of a 40-year-old man who was of short stature, had a round face, short right metacarpal bones III, IV and V, bone formation at the muscle insertions, no calcification of the basal ganglia, bilateral cataracts, normal serum calcium and inorganic phosphorus levels and a sluggish response to intravenous parathyroid extract.

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DETERMINATION IN PLASMA OF FREE 17-HYDROXY AND 17-DESOXY CORTICOSTEROIDS AND THEIR GLUCURONIC-ACID CONJUGATES*

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INTRODUCTION

AS PART of a long-term investigation (1–4) on the systemic effects of both accidental and surgical trauma, it was decided to determine the blood levels of adrenocortical steroids. It is believed that the measurement of the degree and duration of adrenocortical stimulation can be more objectively evaluated by this means than by methods hitherto employed (*e.g.*, urinary excretion studies).

In 1952, Nelson and Samuels (5) reported a method for determining "17-hydroxycorticosteroids" following simple chloroform extraction of plasma or whole blood and chromatographic separation on Florisil, utilizing the Porter-Silber (6) reaction which determines only those steroids containing an alpha-ketol group with a hydroxyl group in the 17 position. In this laboratory it was not found possible to employ this method with any degree of reproducibility. In order to determine adrenocortical steroids reproducibly in peripheral blood plasma, it was found necessary to carry out a more thorough purification of the plasma extracts and to take certain essential technical precautions in the steroid separation and in column chromatography on Florisil or silica. A preliminary report of this method has been given (7); however, in presenting the details, no claims are made for any particular originality. Indeed, well tried extraction procedures were used, as described in the literature (8–11). The procedure of column chromatography employed was essentially that used by Nelson and Samuels, except that certain technically essential features were worked out as to the type of column and the purity and particle size of the Florisil.

Since it is obviously important to study adrenocortical activity as completely as possible, we decided to measure steroids other than the 17-

Received for publication October 27, 1954.

* This work was partially supported by Grant RG-2408 from U. S. Public Health Service and a grant from the Mead Johnson Company.

hydroxycorticosteroids. In addition, therefore, to the Porter-Silber reaction (*see* section IV A) we employed the Mader-Buck blue tetrazolium reduction (12) (*see* section IV B), and the Gornall-MacDonald procedure (13) (*see* section IV C).

Since the Florisil chromatography technic does not separate the 17-hydroxy from the 17-desoxy corticosteroids, it would obviously be preferable to employ a physical separation of the two groups of corticoids before applying the Porter-Silber, the blue tetrazolium and the Gornall-MacDonald reactions. Sweat (14) has described a procedure involving a fractional alcohol-chloroform elution from a silica-gel adsorption column system and claims the ability to separate compounds F_k and B_k consistently. Using the published procedure, together with a personal communication from Dr. Sweat, we carried out twenty experiments involving the separation of various quantities of F_k and B_k. We were successful in effecting a complete separation two out of the twenty times, but were unable to obtain consistent or predictable results as to the degree of separation. We are convinced that under certain possible, but extremely rigid empirical physical conditions, F_k and B_k can be separated by the method of Sweat. However, we feel that this method does not offer promise as an investigative or diagnostic tool because precise consistent control of adsorption chromatography is virtually impossible.

We then turned to the use of a silica partition chromatographic technic similar to that employed by Haines *et al.* (15, 16) for the separation of corticosteroids in adrenal gland extracts. We made one major modification —the use of formamide in lieu of ethylene glycol as a stationary phase. The reason was that we later wished to apply the Norymberski sodium-bismuthate oxidation method (17) to our various fractions and this procedure is not possible in the presence of traces of glycols. We also found that small quantities of ethylene glycol would show a positive reaction with both the Porter-Silber and the Mader-Buck blue tetrazolium methods and, therefore, interfere in quantitative estimations of the various steroids. Removal of these traces of ethylene glycol by vacuum distillation was unsuccessful because the distillation itself formed products of unknown character which also interfered with these spectrophotometric methods of quantitative estimation.

An adequate description of this procedure and discussion of our results are contained in the body of this paper. Using this partition chromatographic technic, complete separation of F_k and B_k can be consistently obtained.

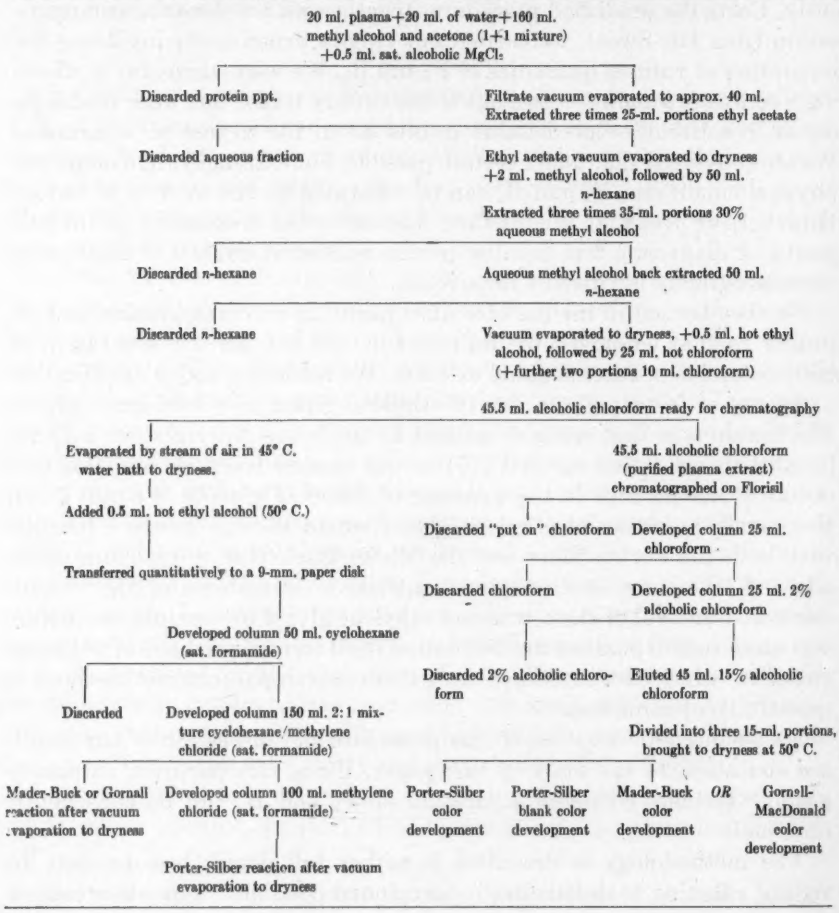
The methodology is described in rather full detail, because only by rigidly adhering to details has it been found possible to reproduce consist-

ently the degree of accuracy in recovery and replicability necessary for the full use of adrenocortical steroid blood values in the assessment of adrenocortical activity.

METHODS

The methods employed are described under six subheadings as indicated below. A schematic "Flow Sheet" of the extraction, chromatographic and spectro-photometric procedures is shown in Table 1.

TABLE 1. SCHEMATIC "FLOW SHEET" OF DESCRIBED PROCEDURES


20 ml. plasma+20 ml. of water+160 ml. methyl alcohol and acetone (1+1 mixture) +0.5 ml. sat. alcoholic MgCl2
Discarded protein ppt.
Filtrate vacuum evaporated to approx. 40 ml. Extracted three times 25-ml. portions ethyl acetate
Discarded aqueous fraction
Ethyl acetate vacuum evaporated to dryness +2 ml. methyl alcohol, followed by 50 ml. n-hexane Extracted three times 35-ml. portions 30% aqueous methyl alcohol
Discarded n-hexane
Aqueous methyl alcohol back extracted 50 ml. n-hexane
Discarded n-hexane
Vacuum evaporated to dryness, +0.5 ml. hot ethyl alcohol, followed by 25 ml. hot chloroform (+further two portions 10 ml. chloroform)
45.5 ml. alcoholic chloroform ready for chromatography
Evaporated by stream of air in 45° C. water bath to dryness.
Added 0.5 ml. hot ethyl alcohol (50° C.)
Transferred quantitatively to a 9-mm. paper disk
Developed column 50 ml. cyclohexane (sat. formamide)
Discarded
Developed column 150 ml. 2:1 mixture cyclohexane/methylene chloride (sat. formamide)
Mader-Buck or Gornall reaction after vacuum evaporation to dryness
Developed column 100 ml. methylene chloride (sat. formamide)
Porter-Silber reaction after vacuum evaporation to dryness
45.5 ml. alcoholic chloroform (purified plasma extract) chromatographed on Florisil
Discarded "put on" chloroform
Developed column 25 ml. chloroform
Discarded chloroform
Developed column 25 ml. 2% alcoholic chloroform
Discarded 2% alcoholic chloroform
Eluted 45 ml. 15% alcoholic chloroform
Divided into three 15-ml. portions, brought to dryness at 50° C.
Porter-Silber color development
Porter-Silber blank color development
Mader-Buck color development
OR
Gornall-MacDonald color development

I. Reagents

Note: All solvents used in the present methods are redistilled from an all-glass Vigreux fractionation apparatus.

1. Methyl alcohol, A.R.
2. Acetone, A.R.
3. Ethyl alcohol, absolute.
4. Chloroform, A.R.
5. Ethyl acetate, A.R.
   An excess of powdered anhydrous $Ca(OH)_2$ A.R. is added before redistillation. Recovery of this solvent is possible after drying over anhydrous, powdered sodium sulfate, A.R., and redistillation as described.
6. *n*-Hexane (Skellysolve B), 68–70° C. fraction.
7. Cyclohexane (practical) (Matheson, Coleman & Bell, Inc.), 80–81° C. fraction.
8. Methylene chloride (Matheson), 40–42° C. fraction.
   It has been found necessary to treat the methylene chloride in the following manner before fractional distillation: the methylene chloride is poured through an activated silica gel (Davison Chem. Co. #200, mesh) column (2″×8″, fritted glass supported). We have found that such a column of activated silica gel is sufficient for at least 4 liters of methylene chloride before retreatment by heating at 600° C. for reactivation.
9. Formamide, C.P.
10. Magnesium chloride, A.R. ($MgCl_2 \cdot 6H_2O$).
11. Dow-Corning Antifoam A spray.
12. Sodium sulfate, A.R., anhydrous, fine crystals, low nitrogen.
13. Ethyl alcohol, absolute. For use in the Mader-Buck reaction, it is treated as follows: to 1,000 ml. of alcohol are added 25 mg. of blue tetrazolium (BT) dye and then 5 ml. of saturated alcoholic potassium hydroxide. The solution is well mixed by shaking and allowed to stand overnight. It is redistilled as usual, 78–79° C. fraction.
14. Florisil, 200/300 mesh (Floridin Co., Warren, Pa.). Two portions of approximately 50 Gm. each of Florisil are transferred into 43×123 mm. Whatman extraction thimbles. Into one all-glass jointed Soxhlet apparatus are placed approximately 250 ml. of absolute ethyl alcohol and into the other Soxhlet, approximately 250 ml. of chloroform, A.R. The apparatuses are heated so that a rate of approximately 5 extractions per hour is attained. The extraction is continued for twenty-four hours. The thimbles are then removed, interchanged and re-extracted for another twenty-four hours. The thimbles are then dried in an electric oven at approximately 150° C. for four hours. The Florisil is removed and stored in a tightly closed bottle. The thimbles are reusable. It is recommended that a duplicate column blank be run before using each batch. An allowable blank with the procedure described subsequently should have an optical density reading less than 0.02 (Porter-Silber) or 0.05 (Mader-Buck).
15. Silica for chromatographic columns, mesh 50/200 (G. Frederick Smith Chem. Co., Columbus, Ohio). It is treated in a manner similar to that described for Florisil (I, 14) but methylene chloride and cyclohexane are substituted for chloroform and ethyl alcohol.
16. Phenylhydrazine hydrochloride recrystallized from absolute ethyl alcohol. M.P. 241° C.

17. Dilute sulfuric acid: to 190 ml. of distilled water are added 310 ml. of conc. sulfuric acid.
    a) Twenty-four mg. of recrystallized phenylhydrazine hydrochloride (I, 16) are dissolved in 15 ml. of dilute sulfuric acid (I, 17). Ten ml. of ethyl alcohol (I, 3) are added and the well mixed reagent is allowed to cool to room temperature.
    b) Fifteen ml. of dilute sulfuric acid (I, 17) are mixed with 10 ml. of ethyl alcohol.
18. Tetramethyl ammonium hydroxide, 10 per cent aqueous solution (Eastman Kodak Co.) titrated with standard acid, using methyl orange indicator. The solution is adjusted to exactly 1.0 normal.
19. Dianisole-bis-4,4′-(3,5 diphenyl) tetrazolium chloride (BT dye, Monomer-Polymer, Inc., Leominster, Mass.). Purification of the relatively crude commercial preparation is accomplished by dissolving 5 Gm. of the compound in 100 ml. of hot (approx. 60° C.) 95 per cent ethyl alcohol. One gram of Norit A (washed activated charcoal) is added and the solution allowed to stand at 60° C. for at least thirty minutes. Filtered through a retentive quantitative type of filter paper, the filtrate is cooled to room temperature. Ethyl ether is added until a dense, light-yellow turbidity results and the material is then placed in a closed flask in the refrigerator overnight. After filtering off at the pump, the recrystallized material is dried in a desiccator over Drierite. Yield: approximately 60 per cent.
    a) Dilute tetramethylammonium hydroxide is prepared by diluting 0.5 ml. of the 10 per cent aqueous solution (I, 18) with 9.5 ml. of 90 per cent ethyl alcohol, prepared from reagent I, 13.
    b) Immediately before use, a dianisole-bis-4,4′-(3,5 diphenyl) tetrazolium chloride (BT) solution is prepared by dissolving 10 mg. (I, 19) in 2 ml. of 90 per cent ethyl alcohol prepared from reagent I, 13.
20. Reagents for *glucuronic-acid hydrolysis:*
    a) Beta-glucuronidase, bacterial origin (Sigma Chem., St. Louis, Mo.)
    b) Aqueous solution (0.5 per cent) of disodium ethylenediaminetetra-acetate (EDTA).
    c) Sorenson phosphate buffer, M/15 pH 6.4.
21. Reagents for *Gornall and MacDonald reaction* (13):
    a) 2,4-Dinitro phenylhydrazine (Eastman Kodak Co. #1866), used without further purification; 0.5 per cent solution in acid methyl alcohol.
    b) Methyl alcohol, A.R., absolute. For use in the Gornall and MacDonald reaction it is treated as follows: to 1,000 ml. of alcohol 2 Gm. of 2,4-dinitro phenylhydrazine are added and the solution allowed to stand for forty-eight hours. It is redistilled as usual, 64–65° C. fraction.
    c) Acid methyl alcohol: to 75 ml. of reagent I, 21(b) are added 25 ml. of concentrated hydrochloric acid A.R.
    d) 4.0 ($\pm$0.05 N) normal sodium hydroxide: prepared from aqueous saturated sodium hydroxide.

II. Plasma extraction

A. *For "free" steroids*

A minimum of 10 ml. (V, 5) of blood plasma is recommended for carrying out this procedure. However, the amounts of solvents and other reagents described subsequently are those used when a 15–25 ml. sample of plasma is employed.

Heparinized plasma is measured into a 250-ml. Erlenmeyer flask and diluted with an equal volume of distilled water. To the flask contents are added slowly, with vigorous magnetic stirring, four volumes of a 1+1 mixture of acetone (I, 2) and methyl alcohol (I, 1) *i.e.*, to 40 ml. of diluted plasma 160 ml. of solvent mixture are added.

At the end of this addition, and with continued stirring, approximately 0.5 ml. of a saturated alcoholic solution of magnesium chloride is added.

The flasks are then placed in an ice bath for approximately one hour. The contents of the flasks are filtered at the pump, using a Coors No. 2 Buchner funnel fitted with a Whatman No. 2 filter paper. The precipitated proteins are discarded and the filtrates are transferred to 250-ml. S/T 24/40 round-bottom Pyrex flasks. Into the neck of each flask is sprayed a very small quantity of Dow-Corning Antifoam A (I, 11). Anti-bumping tubes (V, 4) are placed in the flasks and the vacuum distillation apparatus is assembled as shown in Figure 1 (V, 1). The thermostat of the constant-temperature water bath

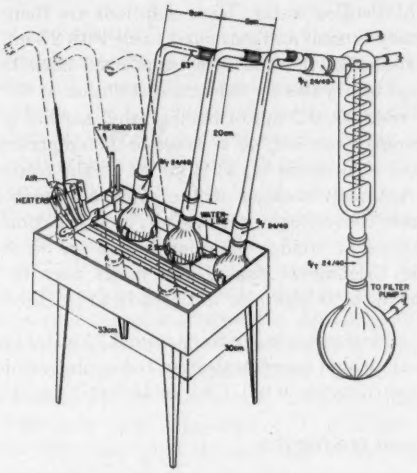


FIG. 1. Apparatus for multiple vacuum distillation at controlled low temperatures. The dimensions given are considered to be optimum using 250-ml. round-bottom flasks containing about 150 ml. of liquid.

(Fig. 1) is regulated for 50° C. A vacuum of approximately 20 mm. of mercury is supplied by the operation of an efficient water pump.

The solvents are distilled off under vacuum and when an approximate volume of 30–50 ml. of liquid remains in the flasks, the vacuum is released and the apparatus dismantled.

The contents of the flasks are transferred into 250-ml. Squibb type separatory funnels and each flask is carefully washed twice with 25-ml. portions of ethyl acetate (I, 5), the washings being transferred to the respective separatory funnels.

The aqueous layer is vigorously extracted and the extraction is repeated twice with 25-ml. portions of ethyl acetate. The ethyl-acetate extracts are collected and pooled in 250-ml. Erlenmeyer flasks and the aqueous fractions are discarded.

To the pooled ethyl-acetate extracts are added approximately 15 grams of anhydrous sodium sulfate (I, 12); the material is mixed well and allowed to stand for about ten

minutes. The supernatant ethyl acetate is decanted back into the respective distillation flasks and the sodium sulfate is washed twice with 10-ml. portions of ethyl acetate. The sodium sulfate is discarded.

The ethyl-acetate extracts are distilled in vacuum to dryness. To the dry residues are added approximately 2 ml. of hot methyl alcohol. The flasks and anti-bumping tubes are then rotated vigorously at a water-bath temperature of 50° C. To the contents of the flasks are then added 50 ml. of *n*-hexane (I, 6). After mixing, the solution is transferred into the respective separatory funnels. The flasks are washed three times with 25-ml. portions of 30 per cent aqueous methyl alcohol, each portion being added to the contents of the respective separatory funnel. After vigorous extracting, the material is allowed to stand about ten minutes, in which time almost total separation of layers has occurred. The partially emulsified aqueous layer is separated and the hexane layer is re-extracted twice with 25-ml. portions of 30 per cent methyl alcohol. The hexane layer is discarded. To each of the combined aqueous methyl-alcohol fractions are added approximately 10 ml. of distilled water. These solutions are then transferred back into their respective separatory funnels and extracted twice with 25-ml. portions of *n*-hexane.

The aqueous methyl-alcohol fractions are transferred back to their respective distilling flasks and reduced to dryness by vacuum distillation at 50° C.

To each of the dry residues, 0.5 ml. of boiling ethyl alcohol is added and the flasks and contents are vigorously rotated, at a water-bath temperature of 50° C. Then a 25-ml. portion of boiling chloroform (I, 4) is added to the contents of each flask and again the flasks are vigorously rotated at the same temperature. The contents are transferred quantitatively into Erlenmeyer flasks. The distillation flasks are washed out twice with 10-ml. portions of boiling chloroform, and the washings then transferred into the corresponding Erlenmeyer flasks. The flasks may be securely closed with aluminum foil and kept in the refrigerator until ready to be chromatographed.

Note: Chloroform, A.R., Mallinckrodt, contains from 0.75 to 1.2 per cent ethyl alcohol as a preservative. The final alcohol concentration of the pooled alcoholic chloroform washings will amount to approximately 0.9–1.1 ml. in 45.5 ml., *i.e.*, 2–2.4 per cent.

B. *For glucuronic-acid conjugates*

Ten to 20 ml. of heparinized plasma are placed in a 250-ml. Erlenmeyer flask and 0.5 ml. of EDTA solution (I, 20 b) and an amount of phosphate buffer (I, 20 c) equal to the volume of plasma being used, are added.

The pH of the diluted plasma should be approximately 6.5–6.8. The flask is placed in a constant-temperature water bath at 37°C. and 750 units (Sigma) of β-glucuronidase (I, 20 b) are added. After incubation for one hour, 750 units of the enzyme are again added. Twice more, at one-hour intervals, the addition of 750 units of β-glucuronidase is repeated. After the last one-hour incubation period a total of 3,000 units of β-glucuronidase has been introduced.

After removing from the water bath, protein precipitation is effected as outlined in the method for the determination of "free" steroids. It was found impractical to perform enzymatic hydrolysis after protein precipitation because excessive foaming occurred during the vacuum distillation and slowly breakable emulsions were formed during the subsequent ethyl-acetate extraction. It is advisable to dissolve 3,000 units of β-glucuronidase in 4 ml. of phosphate buffer and then make the various additions by using 1 ml. of the buffered solution each time. The β-glucuronidase buffer solution is kept in the refrigerator during the intervals between additions.

III. Column chromatography (V, 7)

A. *With Florisil*

The chromatography columns (V, 2a) are thoroughly cleaned and rinsed (V, 6). Florisil, 2.5 Gm. (I, 14) is suspended in a beaker with about 5 ml. of chloroform. This slurry is poured into the column and the walls of the column are washed down with successive small volumes of chloroform until all of the Florisil is tightly packed in the lower end. The length of the Florisil column is approximately 7 cm. When the last portion of the chloroform wash reaches the upper level of the Florisil, the chloroform solution of the plasma extract is poured into the column, being careful that the solution goes down the walls of the column without disturbing the surface of the tightly packed Florisil. When the level of the chloroform solution again almost reaches the surface of the Florisil, the flask which contained the plasma extract is rinsed with approximately 25 ml. of chloroform and this is poured into the column, again taking the foregoing precaution.

The columns are first eluted with 25 ml. of 1 per cent (by volume) alcoholic chloroform (this 1 per cent of alcohol is in addition to the approximate 0.75–1.2 per cent that the A.R. chloroform contains as a preservative) and finally with 45 ml. of 15 per cent (by volume) alcoholic chloroform. The two chloroform fractions and the 1 per cent alcoholic chloroform eluent are discarded. The 15 per cent alcoholic chloroform eluent is collected in a 50-ml. graduated glass-stoppered cylinder. The final volume is made up to 45 ml. with chloroform and thoroughly mixed. Two 15-ml. aliquots are measured into 40-ml. centrifuge tubes, and the third portion is poured into a glass-stoppered centrifuge tube (V, 3). All centrifuge tubes are placed in a 50° C. thermostated water bath in a hood and the chloroform-alcohol mixture is blown off with carefully filtered compressed air. If color development is not to be proceeded with immediately, the open tubes are protected by covering with aluminum foil and placed in the refrigerator.

Together with each series of plasma determinations, it is highly recommended that a recovery be carried out on a previously determined sample of pooled normal blood plasma. This is accomplished by the addition of a known quantity of an alcoholic solution of the pure alcoholic form of compounds E, F or S. Recoveries of compounds A, B and Doc have been determined, but the only suitable method is the Mader-Buck reaction.

B. *With silica*

In a glass mortar are mixed 5 Gm. of silica (I, 15) and 2.5 ml. of formamide (I, 9) previously saturated with both cyclohexane and methylene chloride. A paste is formed, which is then worked in the mortar for ten minutes until it becomes of almost dry-powder consistency. A 15-ml. portion of methylene chloride, previously saturated with formamide, is poured into a clean and dried chromatography column and the stopcock (V, 2 b) is closed. The silica is then transferred from the mortar into the column. Part of it will sink to the bottom, part of it will possibly remain in the reservoir. Another 15-ml. portion of methylene chloride (saturated with formamide) is poured into the column; the stopcock is opened and the walls are washed down. All the silica will sink to the bottom. Using a thick glass rod, of a diameter approaching that of the internal diameter of the column, the silica is packed tightly, taking care that sufficient solvent is at all times above the surface of the silica column. No air pockets should have formed in the process; however, with this manner of packing, there should be no difficulty with air pockets. The length of the silica column should be approximately 10 cm. When the

methylene chloride reaches the upper level of the silica column, taking care that the latter does not dry out, 20 ml. of cyclohexane (also saturated with formamide) are poured carefully down the sides of the column in two portions, being careful not to disturb the surface of the silica. When the cyclohexane reaches just above the upper level of the silica column, the stopcock is closed. The column is now ready for the plasma extract.

The dry plasma residue is dissolved in 0.5 ml. of hot (50° C.) ethyl alcohol. A disk of slightly smaller diameter (approximately 8 mm.) than that of the glass column is cut out of a new or discarded extraction thimble (I, 14) and placed over a 6-mm. hole drilled into a transite plate which is placed over an infrared lamp. With a micropipette the chloroform solution of the plasma extract is slowly transferred onto the paper disk, taking care that the rate of addition does not exceed that of the evaporation of the solvent. When all of the alcohol solution containing the plasma extract has been transferred to the filter-paper disk, the tube which contained the plasma extract is rinsed twice with 0.1-ml. portions of hot alcohol and the washings are also quantitatively transferred to the paper disk in the manner described. The dry filter-paper disk is then placed in the column with the aid of the glass rod previously mentioned and is pushed closely to the surface of the silica column. Fifty ml. of cyclohexane (saturated with formamide) are poured into the column and the rate of elution is regulated to 3 ml. per minute with the stopcock. With a tightly packed silica column it may be even necessary to apply a slight pressure on top of the column from a tank of nitrogen. The cyclohexane is collected in a 50-ml. graduated cylinder.

Just before the cyclohexane reaches the surface of the silica column, the elution of the column is continued with 150 ml. of a 2:1 mixture of cyclohexane:methylene chloride prepared by mixing 100 ml. of cyclohexane (saturated with formamide) and 50 ml. of methylene chloride (saturated with formamide). This eluent is collected in a 250-ml. glass-stoppered Erlenmeyer flask. The rate of flow is maintained at 3 ml. per minute. When the last portion of the solvent mixture reaches the upper level of the silica, the foregoing elution is continued with 100 ml. of methylene chloride (saturated with formamide), collecting the eluate in a 100-ml. glass-stoppered graduated cylinder. The three eluents are each mixed well and divided into three aliquots, then transferred to glass-stoppered 40-ml. centrifuge tubes (V, 3). All centrifuge tubes are placed in a 50° C. thermostated water bath in a hood and the solvents blown off with carefully filtered compressed air. The tubes of the second and third eluents contain a small amount of formamide that cannot be removed by the compressed air. In order to remove the formamide, the tubes are placed in a wire rack inside a large vacuum desiccator (inside diameter 250 mm.). The desiccator is lowered into a constant-temperature water bath at 50° C. and a vacuum is applied from a vacuum pump fitted with a solid carbon dioxide-methyl cellosolve trap. The formamide is removed by distillation in about ninety minutes.

Two out of the three tubes in each set of eluents are used for the Porter-Silber spectrophotometric procedure. The third tube is used for the Mader-Buck reaction.

IV. Spectrophotometric procedures

A. *Porter-Silber reaction* (6)

To one of the open-tube aliquots of the dried plasma eluates prepared by either of the foregoing chromatographic procedures, 0.5 ml. of Porter-Silber reagent (I, 17 a) is added and the tube well rotated to bring the reagent into contact with all the dried material. To the other open aliquot tube is added 0.5 ml. of blank reagent (I, 17 b).

The tubes are placed in a water bath at 60° C. for one hour, and the contents mixed by rotating the tubes from time to time. At the end of the incubation period the tubes are allowed to stand at room temperature until cool. The contents of the tubes are then transferred to suitable cuvettes (V, 5) for spectrophotometric determination at 410 mμ.

The optical density of the plasma tube containing Porter-Silber reagent is read against the corresponding plasma blank tube. An optical density-concentration standard plot is made, using the free alcohol of hydrocortisone (Compound F_k) at concentrations of 0.5–10.0 micrograms per 0.5 ml. of total volume.

B. *Mader-Buck reduction reaction* (12)

The dried eluent contained in the third, glass-stoppered aliquot tube is dissolved in 0.10 ml. of the BT dye solution (I, 19 b). Absolute ethyl alcohol, 0.30 ml. (I, 13), is added and the tubes are incubated for twenty minutes at 37.5° C. in a constant-temperature water bath. At the end of this incubation period, 0.20 ml. of dilute tetramethyl-ammonium hydroxide (I, 19 a) is added and again the tubes are incubated for twenty minutes at 37.5° C. A reagent blank is prepared in identical fashion.

Two standards of both compounds F_k and B_k, *i.e.*, 2 and 4 micrograms of compound F_k, or 2 and 4 micrograms of compound B_k (these amounts are contained in 0.3 ml. of absolute alcohol), are run simultaneously with each set of plasma determinations. Although in our experience the reproducibility of the standard F_k and B_k curves are within ±1 per cent, we recommend that standard curves be repeated until the analyst demonstrates such consistency in his own experience. This precaution is believed necessary because of the need of absolute cleanliness of glassware.

The reaction mixtures, of 0.6 ml. total volume, are transferred into 10-mm. cuvettes (V, 5) and read in a Beckman D.U. spectrophotometer at 510 mμ against the reagent blank.

The following is the procedure used in calculating plasma values. The amount of Porter-Silber chromogens found in an equivalent amount of plasma is calculated as compound F_k. The Mader optical density (O.D.) value of this amount of compound F_k is subtracted from the total O.D. value of the BT reduction. The remainder is read from a B_k O.D. concentration curve.

Example

Total volume plasma used: 20 ml.
Porter-Silber chromogen value: 2 micrograms F_k in 6.66 ml. plasma.
Two micrograms F_k = 0.28 O.D. (Mader-Buck)
Total Mader-Buck for 6.66 ml. plasma = 0.52 O.D.
0.52 − 0.28 = 0.24 O.D.
0.24 = 1.55 micrograms B_k in 6.66 ml. plasma.
1.55 × 3 × 5 = 23.2 micrograms per 100 ml., calculated as compound B_k

C. *Gornall-MacDonald reaction (micro)* (13)

The dry eluent contained in the glass-stoppered aliquot tube is dissolved in 0.2 ml. of methyl alcohol (I, 22). Then 0.2 ml. of 2,4-dinitro phenylhydrazine (I, 21 a) is added and the contents of the tube mixed well and incubated for ninety minutes in a constant-temperature water bath at 59° C. The tubes are protected from direct light. After removing the tubes from the water bath they are allowed to stand at room temperature for two minutes. With gentle shaking, 0.2 ml. of 4 N sodium hydroxide (I, 21 c) is added to each tube; the contents are then diluted with 1.4 ml. of methyl alcohol (I, 22)

and the whole is mixed well. The tubes are allowed to stand for twenty minutes at room temperature. The contents are transferred to suitable cuvettes (V, 5) and read in a Beckman D.U. spectrophotometer at 475 mμ against a reagent blank treated in identical manner.

Two standard concentrations of either F_k or B_k are run with each set of plasma determinations.

V. Notes

1. Figure 1: schematic diagram of apparatus for vacuum distillation for six determinations. Special manifold and bath supplied by Scientific Products Division, American Hospital Supply Corp.
2. a) Special type sintered-glass plate chromatographic columns with 25-ml. reservoir (dimensions without reservoir, 300×10 mm.).
   b) Same as 2(a), with #4 stopcock (4-mm. bore).
   Both supplied by Scientific Products Division, American Hospital Supply Corp.
3. Centrifuge tubes, glass stoppered, 40-ml. capacity, S/T 14/20.
4. Folin-Svedberg type, such as supplied by Aloe Scientific, #12680.
5. The minimum quantity of 10 ml. of plasma is suggested for use *only* with the Beckman D.U. spectrophotometer fitted with a "pinhole" attachment over the light exit and using 50×5×10 mm. light path micro cuvettes such as supplied by the Pyrocell Corp., 207 E. 84th Street, New York, N.Y.
   For quantities of plasma of 20 ml. or more, the Coleman spectrophotometer, model 6A is suitable, using the semi-micro cuvette adapter No. 6-110 and cuvettes made from 7-mm. Pyrex tubing.
6. The columns are thoroughly cleaned with sulfuric-chromic acid cleaning solution and rinsed thoroughly with tap water and finally with distilled water. Thorough rinsing with water is best accomplished by the use of a filter pump. Before use, the columns should be rinsed thoroughly with a small quantity of absolute alcohol in order to remove the last traces of water from the sintered-glass plate, and finally rinsed with chloroform.
7. During chromatography, contamination (*e.g.*, by dust) should be carefully guarded against, especially if working in a laboratory which is not air conditioned. It has been found that covering the columns with aluminum foil between operations is an efficient safeguard against contamination. Temperature and humidity in the laboratory should be controlled and kept constant in order to ensure maximum recoveries in column chromatography.

VI. Evaluation of present methods

Under the following subheadings are described a series of experiments dealing with some of the many factors involved in evaluating the present methods.

A. *Replicability and recovery*

In order to determine the replicability of the foregoing procedures, a sample of normal human plasma was split into six aliquots and stored in the frozen state. On six different days, one of the aliquots was included in the usual run of six determinations. The results of these experiments, as

TABLE 2. REPLICATE DETERMINATION OF STEROID LEVELS IN HUMAN PLASMA

Amt. of plasma	Free "17-hydroxy-corticosteroids' (μg./100 ml.)	"17-hydroxycorti-costeroid" glu-curonic-acid conjugates (μg./100 ml.)	Free "17-desoxy-corticosteroids" (μg./100 ml.)
1) 10 ml.	9.0	18.0	8.5
2) 10 ml.	9.5	16.5	8.0
3) 10 ml.	9.0	17.0	9.0
4) 20 ml.	8.5	19.5	9.0
5) 20 ml.	9.2	16.8	8.7
6) 20 ml.	9.0	17.8	9.0

presented in Table 2, show a replicability of about ±1.0 microgram per 100 ml. for "free" 17-hydroxycorticosteroids and "free" 17-desoxycorticosteroids, and ±2 micrograms per 100 ml. for 17-hydroxycorticosteroid glucuronic-acid conjugates. Although not remarkable from the standpoint of quantitative analytical accuracy, these findings are more than adequate to demonstrate valid changes in a dynamic biologic system. For example,

TABLE 3. RECOVERY OF COMPOUNDS F_k AND B_k (FREE ALCOHOLS) ADDED TO 20 ML. OF PLASMA

Amt. of compound added (micrograms)	Porter-Silber, amount recovered (F_k)		Mader-Buck, amount recovered (B_k)	
	Micrograms	Per cent	Micrograms	Per cent
1	0.90	90	1.0	100
2	1.70	85	1.70	85
4	3.50	87.5	3.60	90
5	4.40	88	4.5	90
5	4.55	91	4.25	85
5	4.3	86	4.3	86
8	6.4	80	6.9	85
10	8.05	80.5	8.8	88
10	8.25	82.5	8.3	83
10	8.65	86.5	8.35	83.5
10	8.65	86.5	9.0	90
10	8.45	84.5	9.3	93
10	8.5	85	9.1	91
		Range: 80–90% *Average:* 86%		*Range:* 83–100% *Average:* 88%

even in normal humans, our values for diurnal determinations vary from 6 micrograms of free 17-hydroxycorticosteroids (17-OH-CS) per 100 ml. at 1 A.M. to 18 micrograms per 100 ml. at 8 A.M. In Table 3 are presented the results of the recoveries following the addition of various amounts of compounds F_k and B_k to normal human plasma. These findings in 26 representative experiments carried out on different days provide additional confirmation to the results presented in Table 1, indicating that the described procedures are interpretable to a limit of at least ± 10 per cent.

B. *β-Glucuronidase hydrolysis*

Since we did not have available a sample of a corticosteroid glucuronate we were forced to establish our conditions of β-glucuronidase hydrolysis on

TABLE 4. PERIPHERAL PLASMA VALUES IN FASTING MAN

Method	Normal subjects	Chronic illnesses and malignancies
Porter-Silber		
No. of subjects	42	50
Average (μg./100 ml.)	9.5	19.5
Standard deviation	1.5	4.3
Mader-Buck minus Porter-Silber		
No. of subjects	29	45
Average (μg./100 ml.)	8.9	14.5
Standard deviation	2.0	3.8
Glucuronic-acid conjugates (Porter-Silber)		
No. of subjects	17	20
Average (μg./100 ml.)	16.8	18.6
Standard deviation	1.6	6.0

a pool of normal human plasma. The conditions studied were: 1) *Optimum pH*—This was found to be the same as that established by Buehler *et al.* (18, 19), namely, 6.5–6.8. 2) *Time of incubation*—This period varied from one to fifteen hours. A safe period in which maximum yield of Porter-Silber chromogens could be obtained was from two to four hours. Therefore, a four-hour period was adopted. 3) *Minimum quantity of enzyme*—For a 20-ml. sample of the plasma pool, the quantity of enzyme varied from 1,500 to 12,000 units (Sigma Chem. Co., St. Louis, Mo.). It was found that 2,250 units was the smallest amount which gave a maximum yield of Porter-Silber chromogens. We, therefore, decided on 3,000 units as a safe amount of enzyme for routine use. 4) *Other conditions*—We were informed by Dr.

Ralph A. Kinsella, Jr., of the Department of Biochemistry, St. Louis University, that the enzyme is rapidly inactivated in the presence of urine at pH 6.5. Therefore, the additions of β-glucuronidase to our plasma samples were made in 750-unit portions at intervals of one hour following the first addition. We were also informed by Dr. Philip A. Katzman of the Department of Biochemistry, St. Louis University, that he had observed certain urine samples which rapidly inactivated β-glucuronidase and that the addition of a small amount of disodium ethylenediaminetetra-acetate (EDTA) greatly retarded this inactivation. We ran three experiments on different samples of blood plasma (20 ml.), with and without the addition of 5 mg. of EDTA. Two of the samples with EDTA yielded approximately 15 per cent more Porter-Silber chromogens than did samples without EDTA addition. We, therefore, included this step in our procedure. In Table 4 the values for normal and abnormal plasmas are presented. Our results do not differ greatly from the few results reported by Bongiovanni (20). Some studies we have made on the use of the Mader-Buck reaction for measuring the hydrolysis products of the β-glucuronidase procedure indicate that there are present in plasma conjugates which, on β-glucuronidase hydrolysis, cause a marked increase in blue tetrazolium (BT) reduction. These conjugates seem to be much more resistant to hydrolysis than those which give rise to an increase in Porter-Silber chromogens. For example, a pooled plasma which had a 17-desoxycorticosteroid value of 12 micrograms per 100 ml., on hydrolysis with 3,000 units of β-glucuronidase (under our standard conditions using 20 ml. of plasma) had a value of 30 micrograms per 100 ml. With 6,000 units of the enzyme, the value was 43 micrograms, and with 24,000 units it was 78 micrograms per 100 ml. We are attempting to characterize these hydrolysis products further. Because of the fact that glucuronic acid reduces BT under the conditions used by us, additions of 5 mg. of glucuronic acid were made to 20-ml. plasma samples and the mixtures carried through the described extraction procedures and Florisil chromatography. No increases in BT reduction were observed.

C. *Porter-Silber reaction*

The only significant modification introduced is the use of a combined reagent (I, 17 a) and ethyl alcohol, instead of methyl alcohol as prescribed by Porter-Silber (6). The use of methyl alcohol in the "combined" reagent results in the development of a pink color after a few hours following its preparation.

Nelson and Samuels (5) recommend the use of the Allen formula (21) in correcting for the presence in plasma extract of nonspecific chromogens. This formula assumes that "the absorption curves of the contaminating

TABLE 5. MOLECULAR EXTINCTION COEFFICIENTS

Compound (free alcohol)	in CH_3OH	Porter-Silber (410 mμ)	Mader-Buck (510 mμ)	Gornall-MacDonald (475 mμ)
E_k	Max. 238 mμ 15,500	21,600	29,000	48,600
F_k	Max. 242 mμ 15,800	21,700	29,000	48,600
S_r	Max. 242 mμ 15,000	21,100	31,200	48,800
Tetrahydro-E_k		19,000	27,000	20,000
Tetrahydro-F_k		21,700	30,800	28,000
A_k	Max. 238 mμ 16,500	—	32,300	37,000
B_k	Max. 241–242 mμ 16,700	—	32,100	41,000
DOC	Max. 242 mμ 16,500	—	30,500	30,000

Sources:

Compound E_k: Merck & Co., 9 RD 8353.
F_k: Merck & Co., 52 R 4270.
Tetrahydro E_k: Merck & Co., 52 R 1950.
Tetrahydro F_k: Merck & Co., 53 R 3598.

All samples of steroids from Merck & Co., Inc. were obtained through the courtesy of Dr. Robert Silber.

Compounds S_r, A_k, B_k, and DOC were received through the courtesy of Dr. Vernon R. Mattox, Mayo Clinic, Rochester, Minnesota.

All of the above compounds were twice recrystallized and melting points obtained which agreed with values in the literature.

substances closely approximate a straight line." We concur with the findings of Bayliss and Steinbeck (22) that this has not been verified experimentally and, further, that the use of this formula does not necessarily correct for the color that the plasma extracts produce with the reagent blank (I, 17 b). We correct for nonspecific sulfuric-acid chromogens by running a plasma extract and reagent blank control with each plasma

determination and readings are made only at the point of maximum absorption, *i.e.*, at 410 mμ.

It may be seen in Table 5 that the molecular extinction coefficient (E) of the Porter-Silber chromogens of the free-alcohol forms of compounds E_k, F_k and S_r, tetrahydro E_k and tetrahydro F_k are, within the experimental limits, virtually identical. These results for compounds E_k and S_r are not in agreement with the original results of Porter-Silber (6).

D. *Mader-Buck, blue tetrazolium reduction*

A simple scaling down of the original Mader-Buck (12) procedure to microgram quantities (0.5–5.0 gamma) was carried out. The results were

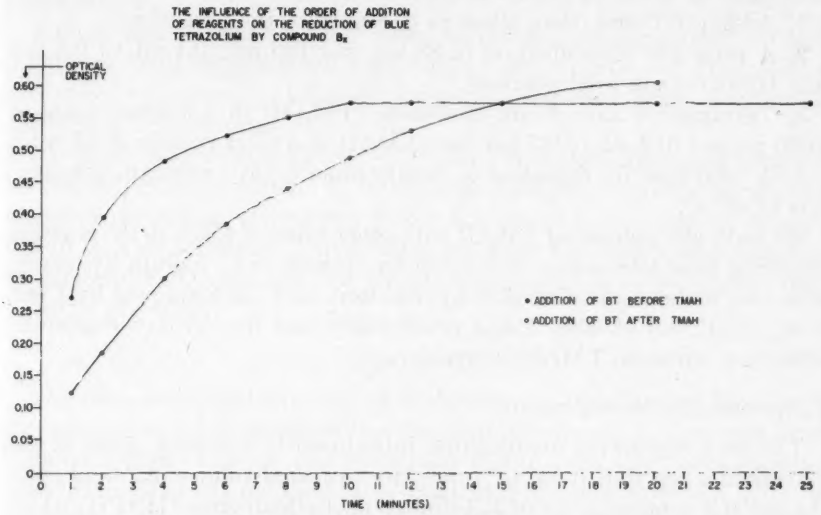


FIG. 2. The influence of the order of addition of reagents on the reduction of blue tetrazolium by compound B_k.

inconsistent and there were marked differences in reduction equivalents with 6 biologically active adrenocortical steroids (E, F, S, A, B, and Doc).

Many experiments were then carried out involving change of reaction conditions, such as dye concentration, alkali type and concentration, temperature and time of reduction, and order of addition of reagents. The following conclusions were reached:

With concentrations of steroid of from 0.5–5.0 micrograms in 0.6 ml. total volume, the most critical condition is the order of addition of blue tetrazolium and alkali. When an alcoholic solution of blue tetrazolium is added to the steroid *before* the alkali, the rate of reduction (Fig. 2) is dis-

tinctly more rapid than when the order of addition is reversed. The steroid reduction equivalents and blank dye values so obtained are completely replicable and reproducible, whereas the values obtained by an opposite order of addition of reagent are inconsistent. We do not completely understand the reactions involved in the reduction of blue tetrazolium in alkali by the steroids and are at present studying the kinetics of these reactions.

On an empirical basis, however, using the following reaction conditions, we have obtained results as regards (a) replicability and day-to-day reproducibility; (b) stability of dye color with time; (c) strict observance of the Beer-Lambert law; and (d) the smallest variation in the reduction equivalents (Table 5) of 6 biologically active steroids tested, as compared with those obtained by Mader and Buck (12):

1. Adding BT first, then alkali as described in the procedure.
2. A total dye concentration of 83 mg. per 100 ml. (0.1 ml. of 0.5 per cent BT in 0.6 ml. total volume).
3. Tetramethyl ammonium hydroxide (TMAH) in a concentration of 0.016 normal (0.2 ml. of 0.5 per cent TMAH in a total volume of 0.6 ml.).
4. A total time for reduction of twenty minutes, at a constant temperature of 37° C.

We have also compared TMAH with other types of alkali in the reaction involving blue tetrazolium reduction by steroids, *i.e.*, sodium hydroxide with and without pyridine plus hydrochloric acid (as employed by Chen *et al.* (23)), and choline. Using replicability and day-by-day reproducibility as a criterion, TMAH is superior.

E. *Gornall-MacDonald reaction*

The only significant modification introduced is a scaling down of the "90 minutes reaction at 59° C" procedure to a total volume of 2.0 ml., plus the use of a concentration of 2, 4-dinitro phenylhydrazine (DNPH) which is half that originally prescribed (13). This change in concentration of DNPH results in halving the blank reagent absorption, without change in the steroid reagent absorption equivalence. A total volume of 2.0 ml. allows the accurate estimation of amounts of 17-hydroxycorticosteroids down to 1 microgram, and of 17-desoxycorticosteroids down to 1.5 micrograms. In Table 5 are presented the molecular extinction coefficients (E) of the DNPH chromogens of the free-alcohol forms of 8 adrenocortical steroids, all in concentrations of 6 micromoles. These values are in fair agreement with those reported by Gornall and MacDonald (13), except that we find no difference in the E values of the "17-hydroxycorticosteroids."

F. *Chromatographic procedures*

Using the column partition chromatographic technic on silica (III, 6),

repeated recovery experiments with F_k and B_k had the following consistent results:

a) The first eluent, cyclohexane, showed none of the two steroids put on the column.

b) In the second eluent (two parts of cyclohexane and one part of methylene chloride), 90 per cent of compound B was recovered, as shown by the Mader-Buck reduction, whereas no compound F could be recovered, *i.e.*, the Porter-Silber reaction was negative.

c) In the third eluent (pure methylene chloride), 90 per cent of compound F_k was recovered as shown by the Porter-Silber reaction. The Mader-Buck reduction also showed 90 per cent recovery calculated as compound F_k.

Similar results were obtained when chromatographing plasma extracts for free steroids on which the Porter-Silber chromogens and Mader-Buck reduction equivalents had been already determined with the Florisil chromatographic procedure.

About 80 per cent of the expected Porter-Silber chromogen was obtained and calculated as compound F_k in the pure methylene chloride eluent, and a similar percentage of recovery in the 2:1 eluent was calculated as compound B_k with a negative Porter-Silber reaction. The results were negative with pure cyclohexane, the first eluent, when tested by either spectrophotometric procedure.

DISCUSSION

The most important question to be discussed is the chemical identity of the substances being measured by the methods described. It would have been preferable to isolate and chemically identify the substances showing the Porter-Silber (6) and the Mader-Buck (12) reaction; however, with the amounts of plasma available, this was not feasible. With larger quantities of material and the development of more precise chromatographic separation, this objective should be possible.

Aside from unequivocal chemical identification, tests for biologic activity of these various fractions would constitute the next best evidence of their adrenocortical origin. Thus far, the facilities for such tests have not been available.

One satisfactory method of determining whether the substances being measured are actually of adrenocortical origin would be observations on a human subject whose adrenals had been removed and who had been maintained for some time without adrenocortical steroid substitution therapy. Such observations have actually been made by us. One patient (M.D.) a 52-year-old woman with metastatic carcinoma of the breast underwent an operation for removal of both ovaries and adrenals. The patient was maintained adequately on cortisone and large doses of sodium chloride. Two

months after operation she was readmitted and kept under close observation for three days, during which period oral cortisone was withdrawn. Within eighteen hours after the last dose, a specimen of blood was obtained. On the third day, just as she showed signs of adrenal insufficiency, a second sample was drawn. Both samples when analyzed contained no Porter-Silber chromogens and there was no reduction of blue tetrazolium. The blood of another similar patient who had been receiving cortisone intramuscularly under similar circumstances showed a zero value for the blue tetrazolium reduction, but continued to show Porter-Silber chromogens, although at much lower levels than before therapy. The difference in this case we presumed was due to the absorption of stored cortisone injected into the muscles.

Another piece of indirect evidence that blue tetrazolium reduction measures steroidal material containing an alpha-ketol group is the finding that the concentration of this material in adrenal venous blood increases approximately in the same proportion over peripheral venous blood as does the concentration of 17-hydroxycorticosteroids. A woman (P.H.) aged 68, during operation, had a peripheral venous blood value of 18 micrograms per 100 ml. for 17-hydroxycorticosteroids and 10 micrograms per 100 ml. for 17-desoxycorticosteroids. During operation, an adrenal venous blood sample was also obtained, in which the concentration of 17-hydroxycorticosteroids was 944 micrograms, and of 17-desoxycorticosteroids, 390 micrograms per 100 ml.

Bush and Sandberg (24) have chemically identified compound F in human plasma and it is now generally believed that a major portion of the Porter-Silber reacting chromogens in plasma extracts is compound F. However, they were unable to show by paper chromatographic technics the presence of substantial quantities of compound B in normal human plasma. Using a paper chromatographic technic (25), we confirmed this observation, *i.e.*, several samples of human plasma showing substantial amounts of blue tetrazolium reducing material (in excess of the amount accounted for by the Porter-Silber chromogen equivalents) failed to show more than a trace of a spot at the compound B position. In order to obtain more information as to the true nature of the substances which react with blue tetrazolium we are at present elaborating the technic for more precise chromatographic separation.

The findings following bacterial β-glucuronidase hydrolysis also deserve comment. The tremendous increase in Porter-Silber chromogens following hydrolysis presumably indicates a release of free 17-hydroxycorticosteroids from their glucuronic-acid conjugates. It is possible that a considerable proportion of this increase is due to release of tetrahydro E or F, an assumption which is based upon the urinary findings of several workers (26, 27). At present we are engaged in the fractionation and identification

of this hydrolyzed material. It is also of interest to note that the increase in blue tetrazolium values following bacterial β-glucuronidase hydrolysis is two to four times greater than the increase in the Porter-Silber chromogens.

In Table 4 are presented the peripheral plasma values in fasting men and women, obtained with the techniques described. The "normal" values were obtained in blood-bank donors, in apparently good health, and in house staff members and laboratory personnel. No consistent variations could be attributed to age or sex. The samples in this group were obtained in the forenoon. The values in the table under "chronic illnesses and malignancies" apply to a diversified group of patients who also showed no consistent variations which could be correlated with their clinical status, age or sex.

SUMMARY

1. A complete description is given of a method of plasma extraction for the partial isolation of the "free" and of the glucuronic-acid conjugated adrenocortical steroids in human blood.

2. Two systems of column chromatographic separation of the adrenocortical steroids in blood are presented.

3. The micro-spectrophotometric determination of the foregoing groups of adrenocortical steroids is described in detail.

4. Experiments are presented concerning the validity of the methodology.

5. Plasma values are presented for "free" and for glucuronic-acid conjugated Porter-Silber chromogens ("17-hydroxycorticosteroids"), together with the values for "free" Mader-Buck minus Porter-Silber chromogens ("17-desoxycorticosteroids"), in "normal" human subjects and in patients who were chronically ill or who suffered from malignancies.

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HUMAN CORTICOSTERONE METABOLISM. V. THE FAILURE OF LARGE ORAL DOSES OF CORTICOSTERONE TO INCREASE URINARY UROPEPSIN

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CORTICOTROPIN regularly increases the urinary excretion of uropepsin. Cortisone induces a similar increase, whether it be given by mouth or parenterally and, as might be expected, hydrocortisone has also been found to increase uropepsin excretion. The effect of corticosterone, the second major component of the normal human response to corticotropin (1), has apparently not been reported. It is of considerable interest, since corticosterone is a steroid with both appreciable glycocorticoid and mineralocorticoid activity and conceivably might reinforce or modify the response to hydrocortisone given at the same time.

SUBJECTS AND METHODS

The studies were conducted with the cooperation of 4 normal young men—medical students or house officers. When oral steroids were given, one-fourth of the total dose was ingested every six hours. Urinary uropepsin was estimated by the method of West and associates (2), and urinary creatinine by the method of Bonsnes and Taussky (3).

The following data illustrate the range of values of the uropepsin/creatinine ratio which may be expected in untreated subjects: In patients

Received for publication October 28, 1954.

* Director, The Unit for Metabolic Research; Armour Fellow in Quantitative Human Pharmacology and Therapeutics, 1953–55.

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‡ Studies of The Unit for Metabolic Research have been supported by grants-in-aid from The Wilson Laboratories, The Armour Laboratories, The Michigan Chapter of the Arthritis and Rheumatism Foundation, and the Medical Research and Development Board, Office of the Surgeon General, Department of the Army under contract DA-49-007-MD-452.

Oral steroids used in this study were made available through the cooperation of Dr. H. F. Hailman and Dr. C. J. O'Donovan of The Upjohn Company and Dr. Elmer Alpert of Merck and Company.

TABLE 1. EFFECT OF ORAL CORTICOSTERONE ON URINARY UROPEPSIN /CREATININE RATIO IN 4 NORMAL ADULTS

(On day 2, each subject received 400 mg. of corticosterone by mouth, 100 mg. every six hours)

Subject	Day 1	Day 2	Day 3
	Control	*Corticosterone*	Control
1	0.34	0.33	0.29
2	0.19	0.07	0.12
3	0.35	0.31	—
4	0.10	0.07	—
Average:	*0.25*	*0.20*	—

with *proven duodenal ulcer* 65 per cent of the ratios were over 0.60, and the remainder were between 0.40 and 0.60. In *non-ulcer patients* 4 per cent of the ratios were over 0.60, 33 per cent between 0.40 and 0.60, and 63 per cent were less than 0.40 (4).

RESULTS

Table 1 shows that, in 4 normal adults, 400 mg. of oral corticosterone did not increase the urinary uropepsin/creatinine ratio. Table 2 shows that, in 1 normal adult, 240 mg. of oral hydrocortisone did induce the expected increase in this ratio, an increase which was not appreciably altered when this amount of oral hydrocortisone was given with 400 mg. of oral corticosterone. The somewhat smaller increase caused by simultaneous ad-

TABLE 2. URINARY UROPEPSIN/CREATININE RATIO AFTER ORAL CORTICOSTERONE AND HYDROCORTISONE SEPARATELY AND IN COMBINATION, IN A NORMAL ADULT MAN

(Steroids were given by mouth as in Table 1. One week elapsed between experiments)

	Day 1	Day 2	Day 3	Day 4
	Control	*Steroid*	Control	Control
400 mg. Corticosterone	0.34	0.33	0.29	—
240 mg. Hydrocortisone	0.35	0.51	0.51	0.30
400 mg. Corticosterone plus 240 mg. Hydrocortisone	0.27	0.45	0.42	0.24
600 mg. Corticosterone plus 120 mg. Hydrocortisone	0.20	0.37	0.20	—

ministration of 120 mg. of oral hydrocortisone and 600 mg. of oral corticosterone suggested that the latter did not antagonize the effect of hydrocortisone on uropepsin excretion.

DISCUSSION

Although corticosterone has one-third to one-half the glycocorticoid activity of cortisone and is well absorbed when given by mouth (5), it is far less than one-third as active as cortisone in its ability to produce eosinopenia (6), to ameliorate rheumatoid (6) or acute gouty arthritis (7) and, from the present data, to increase uropepsin excretion. It would appear probable, therefore, that these characteristic effects of the 11-oxygenated 17,21-dihydroxy-20-ketocorticoids are not importantly related to their effects on carbohydrate metabolism.

Some of the effects of hydrocortisone, notably its ability to increase central excitability, are antagonized by simultaneous administration of corticosterone (8). This, however, does not appear to be true of the antirheumatic, eosinopenic, or uropepsinuric effects of hydrocortisone, and possibly suggests that these actions are not importantly dependent on mineralocorticoid activity. Two practical inferences may be drawn from the lack of antagonism between hydrocortisone and corticosterone on uropepsin excretion: 1) it is improbable that the high uropepsin/creatinine ratio of the person prone to duodenal ulcer is due to a hydrocortisone/corticosterone ratio which is unusually high, and 2) it is unlikely that corticosterone will be valuable in the therapy of ulcer.

SUMMARY

In 4 normal adults, 400 mg. of oral corticosterone did not increase the urinary uropepsin/creatinine ratio. In 1 of these normal adults, the ratio was increased by 240 mg. of oral hydrocortisone, by 240 mg. of oral hydrocortisone with 400 mg. of oral corticosterone, and by 120 mg. of oral hydrocortisone with 600 mg. of oral corticosterone. The antirheumatic, eosinopenic, and uropepsinuric action of corticosterone is either extremely small or absent, which suggests that these effects of hydrocortisone cannot be closely related to its glycocorticoid activity.

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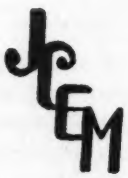

A COMPARISON OF THE THYROIDAL PLASMA I^{131} CLEARANCE AND THE PLASMA PROTEIN-BOUND I^{131} TESTS FOR THE DIAGNOSIS OF HYPERTHYROIDISM

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INTRODUCTION

VARIOUS tests using radioactive iodine for the evaluation of thyroidal disorders have been described in numerous published reports (1–5). The most commonly employed have been the determinations of 24-hour thyroidal uptake (6, 7) or urinary excretion (8, 9) of radioiodine after an orally administered tracer dose. Although excretion measurements are technically easier, there is always uncertainty as to the completeness of the urine collection. The percentage uptake by the thyroid, therefore, has been more generally used. Although a reasonably good distinction between hypothyroidism and euthyroidism is thus obtained, it has become increasingly apparent that the wide overlap in values does not permit a definitive separation between normal and hyperthyroid states.

Most of the tests which have been proposed recently are based on one of two different aspects of thyroid kinetics: the rate of thyroidal removal of iodide from the plasma (4, 10, 11), or the rate of appearance of organically bound iodine in the plasma (8, 12). Wayne (13) and Ansell *et al.* (14) have reported a comparative evaluation of some of these newer methods and indicate that in their experience the determination of the concentration of protein-bound radioiodine in the plasma (PBI^{131}) at forty-eight hours is more satisfactory than either the thyroidal clearance rate or the 4-hour uptake. They emphasize, however, that the 48-hour plasma PBI^{131} level may not reflect the euthyroid status of successfully treated hyperthyroid patients.

This laboratory has compared two of the more definitive radioisotope tests, the thyroidal plasma radioiodide clearance and the PBI^{131} plasma concentration, at forty-eight to ninety-six hours. These tests have been evaluated on the basis of the following criteria: 1) their correlation with

Received for publication December 15, 1954.

* Aided in part by a grant from the Rockledge Foundation.

each other and with the final clinical diagnosis, 2) the influence of previous therapy for thyroid disease, 3) the degree of overlap of values obtained in hyperthyroid and euthyroid states and the significance of that overlap, and 4) the simplicity of the tests and their usefulness in a routine diagnostic laboratory.

MATERIALS AND METHODS[1]

A. *Subjects.* The subjects were hospitalized and ambulatory patients who were referred to the Isotope Laboratory at Lenox Hill Hospital; 27 were males and 109 were females. Ages ranged from 9 to 77 years. Pertinent information regarding the patient's past history, including recent significant medication, was submitted by the referring physician. The size of the thyroid gland was estimated and a tentative diagnosis on the basis of clinical examination was made prior to the radioisotope studies.

The final established diagnosis was based on consideration of the following factors: a careful clinical evaluation, which depended on the case history, physical examination and laboratory data other than radioiodine studies (BMR, and chemical determination of serum PBI and cholesterol levels); the eventual response to therapy or omission of therapy; and the results of radioiodine tracer tests. There was a small group of equivocal cases consisting of patients in whom the final diagnosis was not clearly established because of lack of agreement among these factors. These cases are discussed separately.

B. *Thyroidal plasma iodide clearance.* The thyroidal plasma iodide clearance is defined as the virtual volume of plasma completely cleared of iodide per unit time by the thyroid gland, and is therefore equal to the rate of thyroidal uptake divided by the plasma concentration measured over the same time interval. In this laboratory the thyroidal clearance is determined according to the method described by Berson *et al.* (11). The thyroidal uptake during the first half hour after intravenous administration of a tracer dose is ascertained, making suitable correction for nonthyroidal I^{131} registered by the counter. The average plasma concentration during this period has been shown to be that which would result if the average dose remaining in the body over this time were distributed through a space equivalent to 20 per cent of the body weight. Thyroidal radioactivity was assayed with a bismuth-walled Geiger counter placed 23 cm. from the neck. The sensitivity of this arrangement was 104 counts per minute per microcurie of I^{131}, over a background of 60 counts per minute, as determined by assay of a phantom neck consisting of a beaker filled with paraffin containing a source of I^{131} resembling the thyroid in size and location.

C. *Plasma PBI^{131} concentration.* The plasma PBI^{131} concentration is expressed as the fraction of the administered dose of radioiodine in protein-bound form per liter of plasma. The plasma PBI^{131} levels were usually determined at seventy-two hours after administration of the dose (15). Because this was not always possible, 12 per cent of the determinations were made at forty-eight hours and 26 per cent at ninety-six hours. Since in many cases some $iodide^{131}$ was precipitated with the PBI^{131} by 10 per cent cold trichloracetic acid, an alternative method for determining the plasma PBI^{131} was utilized (16, 17). This method depends on the absence of PBI^{131} in the red blood cells. Since radioiodide concentration in the cells is 0.6 that of plasma, the plasma content

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[1] The radioiodine used in these studies was supplied by Oak Ridge National Laboratory on authorization from the Isotopes Division, U. S. Atomic Energy Commission. It was standardized in the Isotope Laboratory.

of PBI^{131} was determined by subtracting 1.0/0.6 times the I^{131} concentration in packed red cells from the I^{131} concentration in plasma. Measurements were made on 5-ml. samples of red cells and plasma in a well-type scintillation counter with a sensitivity of 1.0×10^6 counts per minute per microcurie of I^{131} and a background of 240 counts per minute.

D. *Procedure.* The average tracer dose, administered intravenously, was between 50 and 60 microcuries. This amount is necessary for the plasma PBI^{131} test and is twice that

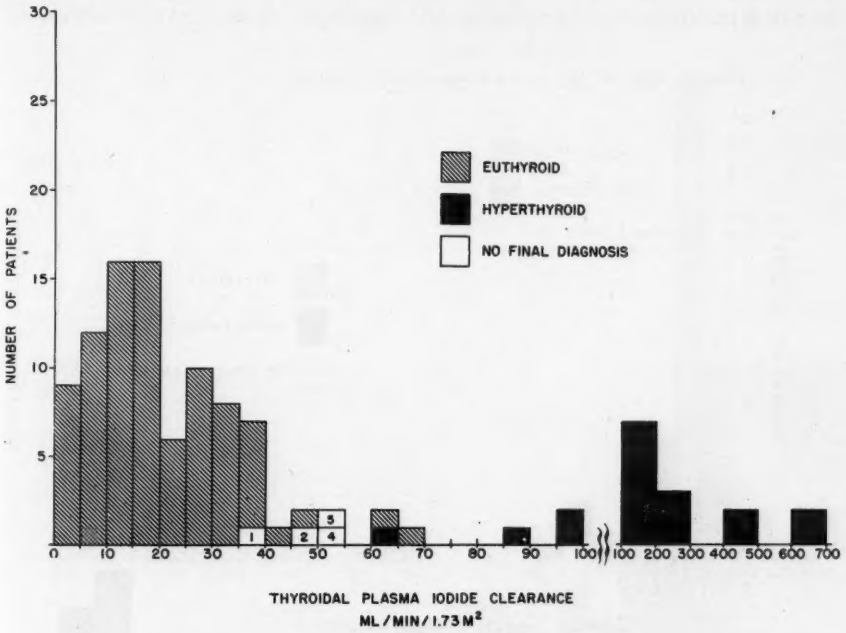

FIG. 1a. Frequency distribution of thyroidal plasma iodide clearance rates in patients previously untreated for thyroid disease.

required for the clearance determination with the equipment employed. Patients receiving either iodine or desiccated thyroid were not tested until three weeks after discontinuing the medication. Antithyroid drugs such as propylthiouracil and methimazole were discontinued at least four days before testing. In a few instances, when it appeared that the findings might have been influenced by the premedication, the tests were repeated after additional time had elapsed. Invariably, results of the later tests were a better reflection of the clinical picture.

RESULTS

A preliminary analysis of the data indicated the advisability of dividing the subjects into two groups, namely, those without previous thyroid therapy and those who had been treated for thyroid disease by surgery

and/or radioiodine. One hundred and fifty-one pairs of tests with I^{131} were completed on 136 subjects. Of the patients tested, 109 had received no therapy directed at the thyroid gland other than desiccated thyroid for suspected hypothyroidism; 87 were euthyroid, 18 were hyperthyroid, and in 4 a diagnosis could not be made. The remaining 42 pairs of tests were performed on 27 patients who had been treated for thyroid disease other than hypothyroidism.

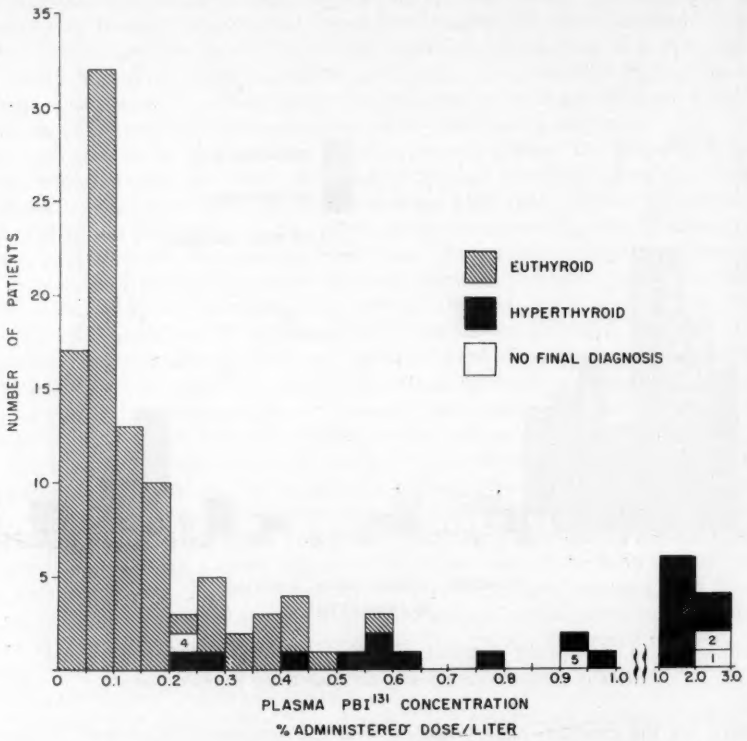

FIG. 1b. Frequency distribution of plasma PBI^{131} concentrations in patients previously untreated for thyroid disease.

Graphs of thyroidal clearances and PBI^{131} levels in previously untreated patients are shown in Figure 1 (a and b). Cross-hatched and solid black columns refer to euthyroid and definitely hyperthyroid subjects, respectively. Numerals are used to indicate the number of patients for whom no final clinical diagnosis was established (Table 1).

It is apparent from an examination of Figure 1a that no euthyroid patients had clearances above 70 ml./min./1.73 M^2 and no hyperthyroid

patients had clearances below 60 ml./min./1.73 M². Accordingly, although 65 ml./min./1.73 M² was chosen as the dividing line, results between 60 and 70 ml./min./1.73 M² were considered indeterminate.

Results of PBI^{131} studies on the same group of patients (Fig. 1b) indicate that no euthyroid patient had a PBI^{131} level above 0.6%/liter. Of the total of 18 untreated hyperthyroid subjects, 6 (33 per cent) had PBI^{131} levels between 0.2 and 0.6%/liter. The wide distribution of results on hyperthyroid individuals precluded the selection of any optimum point of

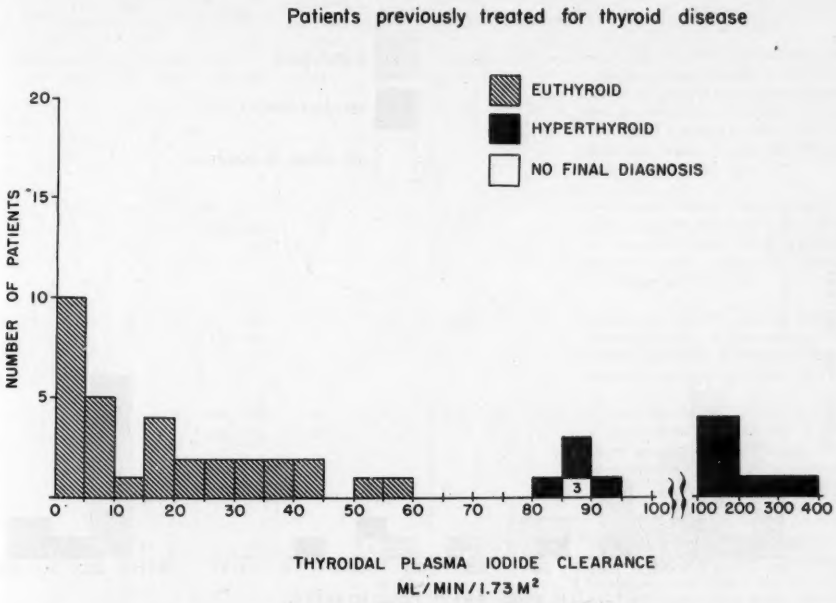

FIG. 2a. Frequency distribution of thyroidal plasma iodide clearance rates in patients previously treated for thyroid disease.

separation. However, the upper limit of normal results (0.6%/liter) was chosen arbitrarily for the point of differentiation.

Graphs of the clearance and plasma PBI^{131} levels in previously treated patients are shown in Figure 2 (a and b). The same symbols are used as in Figure 1. The clearances of all the euthyroid patients in this group (Fig. 2a) were below 60 ml./min./1.73 M² and of all the hyperthyroid patients, above 80 ml./min./1.73 M². In contrast, some PBI^{131} levels in hyperthyroid subjects again appear well below 0.6%/liter (Fig. 2b). Furthermore this graph reveals a marked tendency for clinically euthyroid patients to maintain high PBI^{131} levels after successful treatment of hyperthyroidism.

Graphs of the relationship of thyroidal clearance and PBI^{131} plasma concentration values in untreated and treated patients are shown in Figures 3a and 3b, respectively. Closed circles represent clinically euthyroid subjects, and crosses, clinically hyperthyroid patients. Patients for whom no final diagnosis had been made are indicated by numerals as before. The subjects represented in quadrant A had normal clearances and normal PBI^{131} levels. Patients with clearance rates and PBI^{131} levels in the hyper-

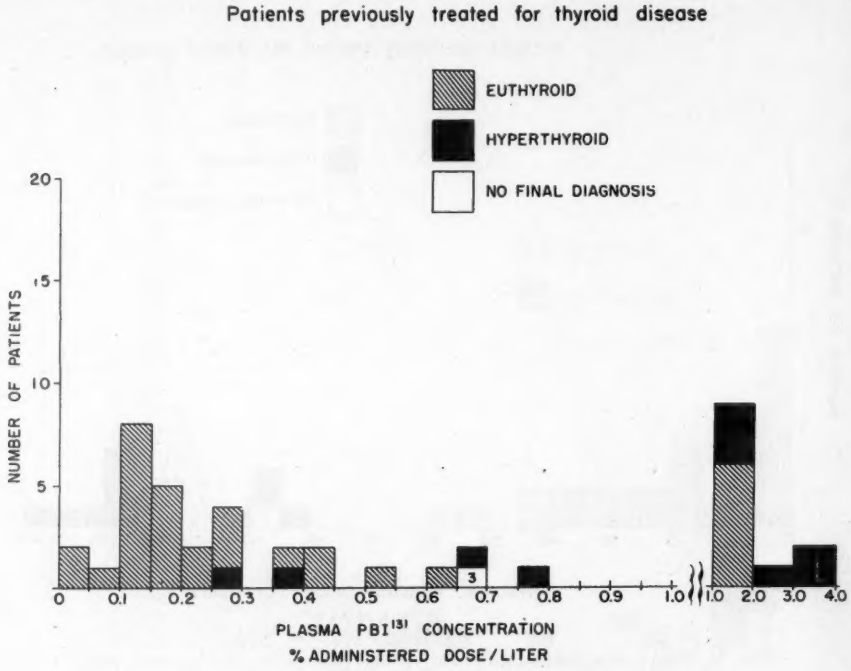

FIG. 2b. Frequency distribution of plasma PBI^{131} concentrations in patients previously treated for thyroid disease.

thyroid range are represented in quadrant C. Of a total of 105 tests on untreated patients with an established diagnosis (Fig. 3a), 6 of the points (6 per cent) lie outside quadrants A and C. Four of these points indicate a discrepancy between the normal PBI^{131} level and the clinical diagnosis of hyperthyroidism and high clearance rate (quadrant D). One point at the intersection of the quadrants represents a hyperthyroid patient. The last discordant result in this group was that of a euthyroid patient with a functioning adenoma who had a normal PBI^{131} concentration and a thyroidal clearance only slightly above the borderline (quadrant D).

Figure 3b shows a much wider scatter of points, with 7 points falling in quadrant B, in sharp contrast to Figure 3a, in which no euthyroid patient had test results falling in this quadrant. The two points in quadrant D indicate clinically hyperthyroid patients with hyperthyroid clearances and euthyroid PBI^{131} levels. Thus in 9 of 42 treated patients (21 per cent) results of the PBI^{131} test failed to correlate with the clinical diagnosis and

TABLE I

No.	Patient	Age (yrs.)	Sex	Date	Thyroidal clearance (ml./min/1.73m²)	Serum PBI^{131} (%/liter)	BMR (%)	Serum PBI (μg./100 ml.)	Remarks
1	H.S.	73	F	May 1953	39	2.5	+31		Lugol's and propylthiouracil in 1948–1949. Always underweight but no recent weight loss or nervousness. Occasional palpitation. Rapid fibrillation. Definite lid lag. Slight fine tremor. Normal palms. Large nodule in right lobe.
2	K.K.	50	F	Dec. 1953 May 1954	49 43	2.19	+42		No previous therapy. Chronic alcoholic with weight loss and tachycardia. Mild, fine tremor. Reg. sinus rhythm, 96. Damp palms. No gland enlargement. Died of hemorrhage and liver failure.
3	W.S.	65	M	Oct. 1953 Apr. 1954	89 64	0.67 0.19	+13, +14	6.8	Subtotal thyroidectomy in 1948. Propylthiouracil in 1951. Mild nervousness. Occasional palpitation. Fluctuating weight. Coarse tremor. Reg. sinus rhythm, 88–108.
4	L.L.	59	M	Feb. 1954 Apr. 1954	51 37	0.23 0.19	+31, +40	9.6	No previous therapy. Always nervous. Weight constant. Slight lid lag. Hyperactive appearance. Aur. fib., rate 104. Marked fine tremor. Wet palms. Enlarged left lobe. Poor response to large doses of digitalis. No response to trial of Lugol's.
5	M.M.	63	M	June 1953 Dec. 1953	53 36 44	0.95 0.76 0.43	+26, +32 +14	5.2	No previous therapy. Weight loss, nervousness, palpitation, fatigue, increased sweating of recent origin. Aur. fib., rate 106. Slight lid lag. Damp palms. Marked fine tremor. Diffuse enlargement, especially of the right lobe. Excellent response to Lugol's.

the thyroidal I^{131} clearance. The thyroidal clearance of I^{131} was consistent with the final clinical diagnosis in all cases.

There were 5 patients of the total studied whose thyroid status was not conclusively determined. The pertinent laboratory data and significant clinical features in these cases are presented in Table 1.

DISCUSSION

In the present state of knowledge of thyroid physiology, probably the most useful laboratory study of thyroid function would be the determination of the rate of secretion of hormonal iodine. If, during the period of

measurement, there is no net change in the distribution or total amount of organic iodine in the body, the rate of secretion is virtually the same as the rate of thyroidal uptake of inorganic iodine and the rate of peripheral utilization of hormonal iodine, since loss of non-utilized hormone in stool and urine is usually small (17). However, information regarding these rates still may not establish the diagnosis of hyperthyroidism. The range of normal values for organic iodine utilization [40–120 μg./day (17)] is such that, if a patient in the low normal range triples his rate of utilization,

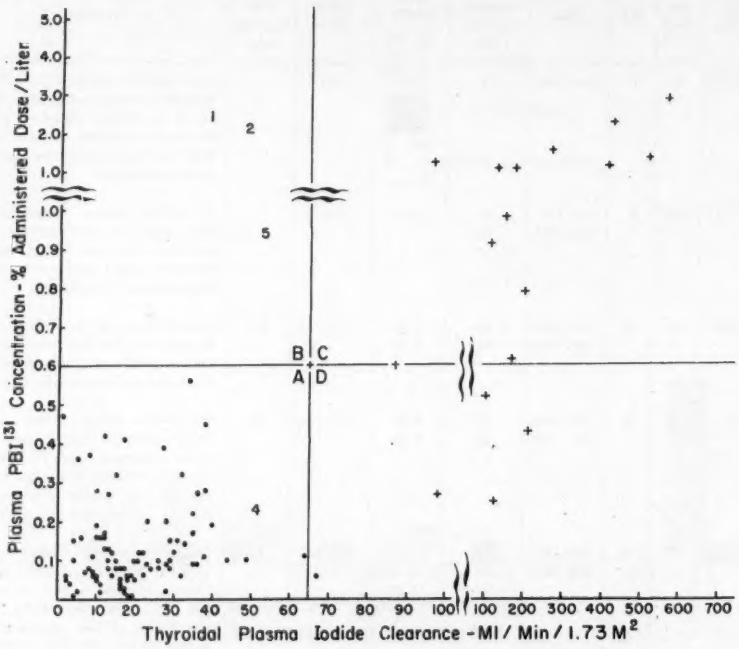

FIG. 3a. Comparison of thyroidal plasma iodide clearance rates with PBI^{131} concentrations in patients previously untreated for thyroid disease.

this new rate, although greatly elevated for him, would appear to be normal. What determines the clinical status of such a patient is an open question. Furthermore, hyperthyroidism itself is characterized by periods of spontaneous remission and exacerbation, and an equilibrium condition with respect to production, release and degradation of hormone may not exist during the period of investigation.

A complete study of the kinetics of synthesis and metabolism of the thyroid hormone is too complicated a procedure to be expedient for routine use, nor is it necessary in the vast majority of the cases presented for

differential diagnosis of hyperthyroidism. Each of the tests evaluated in the present report has its place as a laboratory aid in this differential diagnosis. Since both tests have given results as low as zero for euthyroid patients, it would seem that neither test is suitable for distinguishing hypothyroidism from the normal state.

Maximum usefulness of the thyroidal plasma iodide clearance and the 48–96 hour PBI^{131} tests will be obtained when there is a clear appreciation of the reasons for false diagnostic predictions, the significance of equivocal

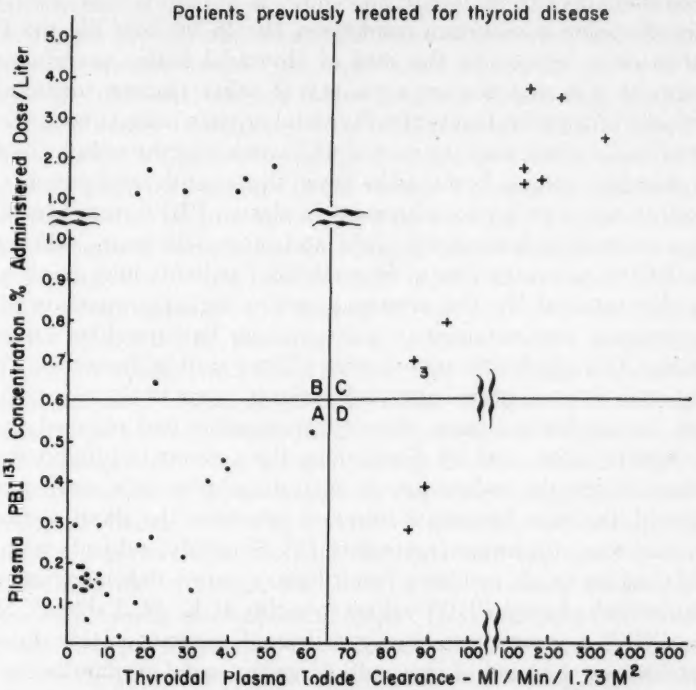

FIG. 3b. Comparison of thyroidal plasma iodide clearance rates with PBI^{131} concentrations in patients previously treated for thyroid disease.

values, and the complications introduced by non-equilibrium states which may follow previous thyroid or antithyroid therapy.

For simplicity of analysis, let us consider first the equilibrium system in which the rate of iodine accumulation by the thyroid is equal to the rate of secretion of hormonal iodine, which in turn is equal to the rate of peripheral utilization of hormonal iodine. At a given plasma iodide concentration, the thyroidal clearance rate is proportional to the rate of daily hormonal iodine synthesis. Since good diagnostic separation was obtained

with the clearance test, it may be inferred that the range of plasma iodide values was not so great as to interfere with the reliability of this test. [K.K. (#2) Table 1 is a possible exception.] It has been observed, however, that when plasma iodide concentrations are very low as a result of dietary iodine deficiency, there is a markedly elevated rate of removal of radioiodide from the plasma, which may persist for at least two weeks after the addition to the diet of 1.5 mg./day of stable iodine (18). Conversely, elevated plasma iodide levels are reflected in a decrease in the thyroidal clearance rate.

Under the same equilibrium conditions, the 48–96 hour plasma PBI^{131} concentration is related to the rate of thyroidal iodine secretion. This measurement also depends on a variety of other factors, including the relative sizes of thyroidal and extrathyroidal organic iodine pools, the peak uptake of radioiodine, and the rate of utilization and the volume of distribution of organic iodine. It should be noted that in euthyroid patients there is almost always a progressive increase in plasma PBI^{131} concentrations in the time interval between forty-eight and ninety-six hours, whereas the plasma PBI^{131} concentrations of hyperthyroid patients may reach a peak during this interval. In the present series, a higher proportion of false positive results was obtained in patients who had previous surgical or radioiodine therapy for thyroid disease (22 per cent of the cases, Figs. 2b and 3b) than in previously untreated patients (none of the cases, Figs. 1b and 3a). In the former cases, therapy presumably had reduced the thyroidal organic iodine pool by diminishing the amount of functioning thyroid tissue. Since the radioiodine is then distributed in a smaller pool in the thyroid, the same hormonal secretion rate from the gland corresponds to the secretion of a larger fraction of I^{131}. Similarly, subjects with small thyroidal iodine pools resulting from dietary iodine deficiency may have spuriously high plasma PBI^{131} values (possibly K.K. #2, Table 1). Normal plasma PBI^{131} concentrations in hyperthyroid patients in this series may be explained on the basis of unusually large thyroidal organic iodine pools, since even a high thyroidal secretion rate of hormonal iodine in such a case corresponds to a smaller fractional output and, therefore, a lower plasma PBI^{131} concentration (17).

It is difficult to correlate the results given by Ansell *et al.* (14) with the analysis presented in this report. They reported 2 nontoxic patients with clearances of approximately 100 ml./min and attributed these elevated rates to iodine-deficient goiters. It is surprising that both patients had 48-hour PBI^{131} concentrations of less than 0.2%/liter, since in the study of Stanbury *et al.* (18) an iodine-deficient subject was shown to secrete daily about 13 per cent of his thyroidal radioactivity. In addition, Ansell *et al.* (14) reported 13 hyperthyroid patients with clearances below 80 ml./min,

including 7 with clearances below 50 ml./min. All of these patients had elevated 48-hour PBI^{131} levels. These results were obtained mainly in patients with toxic adenomatous goiters. The explanation that "the low clearance rate of normal gland tissue 'masked' the higher clearance rate of the adenoma" is not tenable, since it is the overall function of the thyroid gland that determines production of thyroidal hormone. If the plasma iodide concentrations in such patients are normal (and there is no reason a priori to suspect elevation of plasma iodide levels in this group of patients), the rate of uptake of iodide and, therefore, the rate of hormonal iodine synthesis are normal. Increased rates of secretion and peripheral utilization of hormone would thus imply a non-equilibrium state in which the size of the thyroidal organic iodine pool was decreasing. A follow-up on the clinical course of such patients with subsequent radioiodine tests would have been of value in elucidating the interpretation of such findings.

Since many patients examined in the Isotope Laboratory had had desiccated thyroid, iodine or antithyroid drug therapy prior to the radioiodine tests, it is necessary to consider the effect of these therapeutic drugs on the test results. Two euthyroid patients who had received desiccated thyroid for an extended period showed higher thyroidal clearances one month after discontinuing the drug than at a later date, confirming an earlier report by Greer (19). The PBI^{131} level is also likely to be elevated as a result, at least, of the elevated thyroidal uptake. In those patients who had received iodine, elevated iodide levels caused a reduction in the thyroidal clearance of iodine and a lowering of the plasma PBI^{131} level through an inhibition of thyroidal uptake and secretion. After administration of inorganic iodine, two to three weeks is generally sufficient to reduce the plasma iodide levels to normal. The thyroidal clearance is usually reliable at this time, although the effect of the increased plasma iodide levels may occasionally last somewhat longer. In general, the results following organic iodine administration are less predictable, because of continued release of iodide from drugs such as Priodax, which may remain in the body for as long as a year (20). Following prolonged therapy with large doses of antithyroid drugs, the size of the organic iodine pool may be decreased, since these drugs diminish or prevent the synthesis of new hormone. In such cases the plasma PBI^{131} levels may be disparately elevated for a short period following withdrawal of medication. The thyroidal clearance may also be increased, especially if the gland size has increased during the drug therapy. Since the duration of effect of the three different types of drugs cannot be predicted with absolute certainty, repeated testing is usually indicated until thyroid function is stabilized.

Radioiodine tests must also be interpreted with caution in any non-equilibrium state such as may be associated with thyroiditis or the presence

of functioning adenomata. During the acute phase of thyroiditis, the thyroidal clearance and plasma PBI^{131} concentrations are markedly reduced. During the recovery phase both may be elevated before returning to normal. Two such cases were observed in this series, although only the last normal result is shown in Figure 1. A radioiodine test may not reflect the clinical picture of a patient with a functioning adenoma in a temporary non-equilibrium condition. The thyroidal clearances observed in one such patient in this series fluctuated between euthyroid and hyperthyroid values.

Most of the borderline levels in both tests seem to occur in patients whose clinical thyroidal state does not require immediate definitive therapy. Such patients may be in a transitional period, and re-testing usually clarifies the diagnosis. This is particularly true during the months following I^{131} therapy.

In the 5 cases shown in Table 1, a final diagnosis was not established. In only 1 case (#5) was the clinical picture such that therapy seemed to be indicated. Subject H. S. (#1) had a history of symptoms and signs suggestive of hyperthyroidism for several years. The thyroidal clearance was definitely in the normal range and her 72-hour thyroidal uptake was 28 per cent. The greatly elevated PBI^{131} concentration (2.5%/liter) is difficult to explain. If this latter result were valid, it would indicate that about 40 per cent[2] of the patient's organic iodine pool was extrathyroidal. Assuming a chemical PBI level of 8 μg./100 ml., which is in the upper normal range, the total organic iodine pool ($\sim$1600 μg.) was about one-sixth normal. There is nothing in the history to suggest a possible explanation for such a depleted organic iodine pool. No follow-up was available.

Subject K. K. (#2) was a patient with a long history of alcoholism. Her physical signs and symptoms could well be accounted for on this basis. An inadequate, iodine-deficient diet associated with the chronic alcoholism could explain a plasma iodide clearance in the high normal range. Her greatly elevated PBI^{131} level could also be accounted for on the basis of a depleted organic iodine pool. The clinical impression was that this patient was probably not hyperthyroid. Other instances of high thyroidal iodide clearances (21) and uptakes (22) in chronic alcoholics have been observed.

Subject W. S. (#3) was a post-thyroidectomy patient whose thyroid status appeared to fluctuate between a normal and a slightly toxic con-

[2] The extrathyroidal volume of organic iodine distribution in this normal subject was about 10 liters. Hence 2.5%/liter $\times$ 10 liters or 25 per cent of the dose was circulating compared to 28 per cent of the dose still in the thyroid. Since constant specific activity may not have been reached, the lower limit for the extrathyroidal organic iodine pool was 25/25+28 or more than 40 per cent of the total organic iodine pool.

dition. The radioiodine tests and the chemical PBI,[3] determined while the patient seemed in a state of remission, were consistent with this picture.

Subject L. L. (#4) presented a clinical picture suggestive of mild hyperthyroidism, confirmed by the slightly elevated chemical PBI concentrations. However, the lack of response to Lugol's solution and the persistently normal results with tracer tests did not support this diagnosis.

Subject M. M. (#5) was considered hyperthyroid on the basis of the clinical picture and the response to therapy with Lugol's solution. He had shown no response to four weeks' therapy with a daily dosage of 40 mg. of methimazole. The thyroidal clearance and the chemical PBI level were within normal limits. The 72-hour PBI^{131} concentration (0.76% /liter) was only slightly elevated; a higher level, 0.95%/liter was obtained at ninety-six hours. Because of the discrepancy between the clinical picture and the laboratory findings, no certain diagnosis seemed justified; the patient was maintained in good health on Lugol's solution for over one year.

Second in importance to dependability of results in the selection of a tracer test is the practicality of the method. The clearance test is somewhat more cumbersome, requiring the presence of the patient for a period of about forty-five minutes and necessitating intravenous administration of the isotope. The diagnosis is obtained within a few minutes of completion of the test, however, and a second visit by the patient is usually not required. If the results suggest the clinical diagnosis of hypothyroidism, or if they are equivocal or inconsistent with the clinical picture, uptake studies and/or 72-hour plasma PBI^{131} levels can be undertaken, utilizing the same tracer dose. Further, it is comparatively easy to check the results for internal consistency. The PBI^{131} method has the distinct advantage of simplicity. The patient need not come to the isotope laboratory. The physician may administer the dose and collect the blood sample in his office. This method does, however, require a larger tracer dose, a delay of at least two days before obtaining the diagnosis, and a second visit by the patient to a physician or laboratory. In addition, no independent checks are possible, either on the size of the dose administered or on the occurrence of radioiodine contamination in the collected blood specimen.

The observations presented in this report favor the conclusion that the thyroidal clearance of I^{131} is a more reliable measure for thyroidal function than the plasma PBI^{131} concentration in all cases. In previously treated patients, the plasma PBI^{131} concentration is frequently misleading.

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[3] The serum protein-bound iodine level was determined in the Chemistry Laboratory of the Lenox Hill Hospital by the method of Barker *et al.* (23).

SUMMARY

1. The thyroidal plasma I^{131} clearance and the 48–96 hour plasma PBI^{131} concentration tests have been compared with respect to their usefulness for the evaluation of thyroidal function. One hundred and thirty-six patients were studied, including a group of 27 patients who had previously been treated by radioiodine or surgery for thyroid disease.

2. Whereas good differentiation between toxic and nontoxic states was obtained with both tests in previously untreated patients, the plasma PBI^{131} test was frequently misleading in patients previously treated for thyroid disease.

3. An attempt has been made to analyze and interpret the equivocal and discordant results. The effects on thyroid physiology of surgical and radioiodine therapy, premedication with desiccated thyroid or antithyroid drugs, and dietary deficiency or excess of iodine have been discussed.

Acknowledgments

We wish to thank Mr. Paul Newman for the illustrations, and Miss Eve Spelke for secretarial assistance.

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CHRONIC THYROIDITIS AND RIEDEL'S STRUMA: ETIOLOGY AND PATHOGENESIS.

THE ROLE OF THE HYPERFUNCTIONING PARENCHYMA OF PRIMARY HYPERPLASTIC GOITER*

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CHRONIC nonspecific thyroiditis is a nonbacterial inflammatory lesion of obscure etiology, characterized by progressive deterioration and dissolution of the thyroid parenchyma and ultimate replacement by fibrous tissue. The purpose of this report is to submit data in support of our belief that the etiology resides in irritative factors inherent in the hyperfunctioning epithelium of hyperplastic (toxic diffuse) goiter. Moreover, we hope to show that the pathologic process originates in an inflammatory tissue response to these factors which, in turn, effects the degeneration and eventual dissolution of the hyperplastic epithelium. Finally we hope to establish that chronic thyroiditis may progress to its terminal stage of Riedel's struma, which should not in any way be confused with struma lymphomatosa (Hashimoto). The latter is a distinct pathologic entity.

REVIEW OF LITERATURE

In spite of the enormous literature on chronic thyroiditis and Riedel's struma the etiology of both conditions remains completely obscure. Impetus was given to the study of chronic fibrotic lesions of the thyroid by Riedel (1). In 1896 he reported his first 2 cases of the final stage of fibrosis, since designated as Riedel's strŭma. He pointed out the so-called "iron hard" consistency of the thyroid gland and its tendency to form firm adhesions to neighboring cervical structures. In 1897 he presented his third case, on which he made a "follow up" report (2) in 1910. He described the microscopic findings as accumulations of spindle and round cells between the normal elements of the thyroid, which were more and more displaced by the development of young connective tissue. Though the views of some of the early authors are given little or no credence today, they have a historical interest and may be briefly noted.

* Presented at the Annual Meeting of the American Goiter Association, St. Louis, Mo., May 1–3, 1952.

Syphilis was considered a possible etiologic factor. However, evidence for this assumption could not be found (3, 4), spirochaetes were not identified (5) and results of animal inoculations were negative (6, 7). Serologic tests showed absence of syphilis in the cases reported from the literature up to 1932 by Eisen (8). Others also reported negative findings with Kahn (9) and serologic tests (10).

Tuberculosis was formerly suspected because of the chronic nature of Riedel's struma, the lymphocytic infiltration and the frequent occurrence of giant cells. However, stains for tubercle bacilli by Grünberg (5), Vaux (11) and others (12, 13) were not revealing and results of tissue cultures and animal inoculations (4, 6, 13) were also negative. The giant cells in Riedel's struma were thought by some to be indicative of tuberculosis (14–16). Jaffé (17) reported structures resembling miliary tubercles and Vaux (11) described giant cells in 2 cases of "atypical lymphadenoid goiter." The significance of these so-called foreign-body giant cells was finally clarified by Goetsch (18) who demonstrated that they represented nonphagocytic conglomerates of desquamated follicular epithelial cells. These giant cells could therefore not be considered indicative of either tuberculosis or syphilis.

Advanced chronic thyroiditis (Riedel) was assumed by many to be the late result of acute bacterial infection. Confirmatory support was not forthcoming in studies by Clute and Lahey (19), Schilling (10) and others (3, 6, 20). Womack (21) failed to induce inflammatory changes in the thyroid by injections of bacteria into the superior thyroid arteries of dogs and rabbits. Riedel's struma was felt to be a nonspecific granuloma by Ewing (22) and others (23, 24), and a chronic inflammatory condition by many (25–27). Graham and McCullagh (28) stated that Riedel's struma more nearly approaches a true inflammation than does Hashimoto's disease. A history of constitutional infections (29) was not obtainable (6); thus, such infections were not considered to play a part (23). German (30) felt that obliterative vascular changes might act as a cause, and DeCourcy (31) hypothesized that perithyroiditis induced ischemia and a consequent ligneous induration. Supporting evidence for these various views has been lacking.

The striking fibrosis and induration of the thyroid gland in Riedel's struma often led to the clinical diagnosis of suspected malignancy (25, 32, 33). In 2 of the 6 cases reported by Kreuzbauer (12), microscopic examination proved the diagnosis of malignancy incorrect. This diagnosis was made in some instances on the basis of biopsy findings (27, 34), but could not be confirmed by Gilchrist (35) or positively ruled out in the cases reported by McClintock and Wright (26). Operative findings were often confusing and the clinical postoperative studies eventually excluded malignancy (27, 36, 37) in favor of inflammatory fibrosis (3, 4, 38). It is surprising to note the frequency with which the diagnosis of carcinoma was made in the earlier reports. Thus Graham (39) stated that in 90 per cent of authentic cases of Riedel's struma reported up to 1931 a preoperative diagnosis of definite or suspected malignancy had been made. On the other hand Eisen (40) found that 66 per cent of authentic cases reported up to 1932 had been erroneously diagnosed as malignancy, and in 1935 Boyden, Coller and Bugher (9) reported 163 cases (Riedel and Hashimoto) from the literature in which the diagnosis of malignancy had been made in 48 instances. In their own 9 cases, the preoperative diagnosis was carcinoma in 2, probable carcinoma in 1, and adenocarcinoma (by frozen section) in 1. Clute and Lahey (19) in 1932 reported that of their 43 cases of chronic thyroiditis, 3 were considered malignant and 4 possibly malignant. However, at the present time Riedel's struma is generally considered to be a benign lesion. It therefore becomes imperative, though difficult, to differentiate it from malignancy.

Many authors have reflected on the significance of the lymphoid tissue found in both chronic thyroiditis and Graves' disease and have assumed that the lymphocytic infil-

tration in each instance may be caused by the same agent. Stewart and Gibson (41) described the plasma and lymphoid aggregates in the thyroids of Graves' disease, and A. Kocher (42) was the first to emphasize the occurrence of lymphocytosis and lymph follicles. Replacement by lymphoid tissue was believed to be secondary to atrophy of the follicular epithelium induced by iodine (43, 44) or to an irritative hypersecretion similarly induced (6). On the contrary, Simmonds (45) believed that lymphocytic infiltration was the primary cause of the epithelial disintegration and he was probably the first to suggest an etiologic relationship between the hyperfunctioning parenchyma of Basedow's disease and inflammation of the thyroid.

The formation of lymphoid foci was believed by several authors (43, 46, 47) to be caused by the ingestion of iodine, a view to which Boyden, Coller and Bugher (9) inclined. There has been fully as much, or more, support for the view that iodine is not an etiologic factor (6, 12, 42), or certainly not the only factor (44), causing the production of lymphoid tissue. Simmonds (45) found lymphoid tissue in 75 per cent of 120 patients with Basedow's disease, who were not treated with iodine. McCarrison (48) concluded from his experiments on rats that deficiency of vitamin A and not the ingestion of iodine was a factor in producing changes analogous to those of Riedel's struma.

The reaction of the lymphoid tissue was supposed to be secondary to irritative chemical changes in the gland (49) or to an abnormal thyroid secretion (4, 45, 50). Brünger (16), however, considered the inflammation to be the primary cause of the altered secretion. Chronic thyroiditis was also assumed to be secondary to epithelial degeneration (51, 52), to some abnormality of the acinar epithelium per se (53), to the absorption of inflammatory products, to abnormal physiologic demands (54), and finally to atypical involution of the hyperplastic gland (25). Vaux (11) concluded that the microscopic changes could be accounted for by excessive involution following thyrotoxicosis and a superimposition of chronic inflammation. A somewhat similar view had been previously expressed by Williamson and Pearse (55). Confirmatory support for these views was generally lacking.

Hyperthyroidism and "Basedow" findings were etiologically related to chronic thyroiditis by a number of authors and denied by others. Brünger (16) was probably the first to report Basedow symptoms and signs, including mild exophthalmos, in patients with chronic thyroiditis (Riedel's struma). Reference was made to the coexistence of chronic thyroiditis and hyperthyroidism (6, 32), and Boyden, Coller and Bugher (9) specifically reported an instance of Riedel's struma in a woman who was operated upon after having suffered from hyperthyroidism for five years.

An association of hyperthyroidism and an elevated basal metabolism with chronic thyroiditis [Haines (56), Clute and Lahey (19) and others (57, 58)], and the coexistence of this syndrome and prominence of the eyes have been reported by Jackson (59), Rankin and Donald (60) and others (37, 61, 62). Several authors have actually denied this association (13, 63, 64, 65). Means (66) stated that thyroid hyperfunction was not observed in the clinical course of fibrous thyroiditis. A decided difference of opinion has existed as to whether hyperthyroidism may precede or coexist with chronic thyroiditis —a difference which we believe is due largely to the fact that authors have been describing different stages of the same progressive lesion.

The occurrence of hypertrophic and hyperplastic parenchyma in chronic thyroiditis has been commented upon (16, 38, 56, 58, 61). Clute and Lahey (19) regarded the hyperplasia as a result of stimulation by an inflammatory process. The thyroid parenchyma in the early stages of chronic thyroiditis was stated by Boyden, Coller and Bugher (9) to resemble the hyperplastic gland of exophthalmic goiter. The occurrence of isolated nests of hypertrophic thyroid follicles, commonly incarcerated in the hyalinized stroma

of Riedel's struma, was variously interpreted. Thus Kreuzbauer (67) at first considered them as indicative of carcinomatous degeration; Eisen (40) and others (16, 31, 63) believed them to be regenerated compensatory epithelium substituting for the loss of the original thyroid epithelium. Eberts and Fitzgerald (32) felt they represented compensatory epithelium which had survived, and Zelle, Vass and Steer (54) interpreted them as an attempt at regeneration. Schultz (23) stated that parenchymal hyperplasia was no factor in the production of the histologic changes found in Riedel's struma.

Recently Chesky, Dreese and Hellwig (68) have offered the opinion that chronic thyroiditis is a chemical inflammation surrounding macrophages (and their contained colloid) which have disintegrated in the stroma.

It is evident that an extensive review of the literature has failed to reveal the etiology of chronic thyroiditis and Riedel's struma.

MATERIAL AND METHODS

Clinical

With an occasional exception, all the thyroidectomies were performed by one of us (E.G.) at the Long Island College Hospital. In general, our patients had been given the customary preoperative treatment with Lugol's solution. This was during the period before the introduction of the antithyroid drugs. These facts deserve mention, since an appraisal of the former functional activity of thyroid epithelium, which is based on the temporary resting phase of colloid involution or the excessive hyperplasia following treatment with antithyroid drugs (thiouracil and its derivatives), could easily be erroneous.

Histologic

Our material consisted of the resected tissues of diffuse hyperplastic goiters which revealed varying degrees of chronic thyroiditis. Several blocks were cut from each thyroid specimen immediately after removal; these were put at once into fixing solutions. It was imperative to observe sections from multiple blocks, in view of the great variability of the histologic features in different portions of a single thyroid lobe.

Formalin and Zenker's fluid were most frequently employed. On occasion, other fixing fluids were used. Hematoxylin and eosin served for general histologic study; collagenous connective tissue was demonstrated by means of Mallory's connective tissue stain. Plasma cells were stained by the Unna-Pappenhaim technique and elastic fibers were demonstrated by Weigert's elastic-tissue stain.

It was necessary to employ staining methods capable of demonstrating intracellular features which could be used as criteria of cellular hyper- or hypofunction. Such methods are available in Mallory's connective tissue stain, but more particularly in the excellent technique of Bensley (69) for the demonstration of the mitochondria. The latter are tiny, granular or rod-like cytoplasmic constituents occurring in all living cells. They produce enzymes which have an important intracellular oxidizing function. Their numbers are greatly increased over the normal in the hyperfunctioning rapidly oxidizing cell, whereas in the hypofunctioning cell they are distinctly diminished. It was demonstrated by

Goetsch (70) and later confirmed by Key (71), that in the epithelial cells of toxic hyperplastic goiter the mitochondria are present in numbers markedly increased over the normal, whereas in the cells of the hypofunctioning colloid gland the mitochondria are smaller and occur in numbers far below the normal. These experiences were confirmed many times in examining tissues from disordered states of the thyroid gland and consequently we felt secure in establishing an increased or diminished number of mitochondria as criteria of cellular hyperfunction or hypofunction, respectively. We were thus enabled to follow the fate of the hyperfunctioning parenchyma of hyperplastic goiter through the progressive phases of chronic thyroiditis.

Intracellular lipoid bodies are virtually completely absent in the hyperfunctioning epithelium of diffuse toxic goiter, whereas fat globules are common in colloid goiter and are found in abundance in deteriorating hypofunctioning epithelium. Accordingly, intracellular lipoid bodies served as an additional index of epithelial hypofunction. They appeared black after staining with osmic acid and dark brown in tissues fixed in Bensley's acetic-osmic-bichromate mixture. The number of mitochondria bore an interesting inverse relationship to the number of fat globules; as the mitochondria significantly decreased in number the fat globules increased, and vice-versa.

The presence of unusual numbers of lymphoid and plasma cells in an area of parenchyma arrested our attention, since these cells are commonly regarded as indicative of a local irritant. Numerous round cells in an area of parenchyma would accordingly denote marked local irritation, whereas parenchyma in which there is an absence of these cells would be regarded non-irritating.

RESULTS

It would be difficult to report the histo-pathologic findings in a slowly advancing lesion such as chronic thyroiditis, without subdividing the prolonged course of the disease into convenient, though not too sharply demarcated phases. The chronic inflammation was therefore subdivided into four phases, depending upon the extent of epithelial deterioration and the degree of fibrosis: 1) incipient, 2) early, 3) intermediate, and 4) terminal.

1. *Incipient phase*

We chose for special study 10 patients from a large number presenting toxic hyperplastic goiter. There were 4 children under 12 years of age, and 6 adults varying in age up to 49 years. There was nothing in their past histories to indicate acute or chronic infections. They were all females who had symptoms of acute hyperthyroidism over periods of six to twenty months. Three of the patients had exophthalmos. The basal metabolic rates were uniformly elevated and varied between +15 and +42 per cent. The thyroid glands were diffusely and symmetrically enlarged, fairly firm, finely lobulated with increased vascularity, and typical of toxic hyperplastic goiter. Microscopically, all exhibited the characteristics of incipient chronic thyroiditis. The 10 patients were submitted to partial thyroidectomy after preoperative treatment with Lugol's solution.

Macroscopic: Grossly, the excised tissues were characteristic of hyperplastic glands which had undergone involution by iodine. Cut surfaces revealed a minimum of fibrous tissue. Areas suggesting gross infiltration with lymphoid tissue were not noted. The capsules were not thickened.

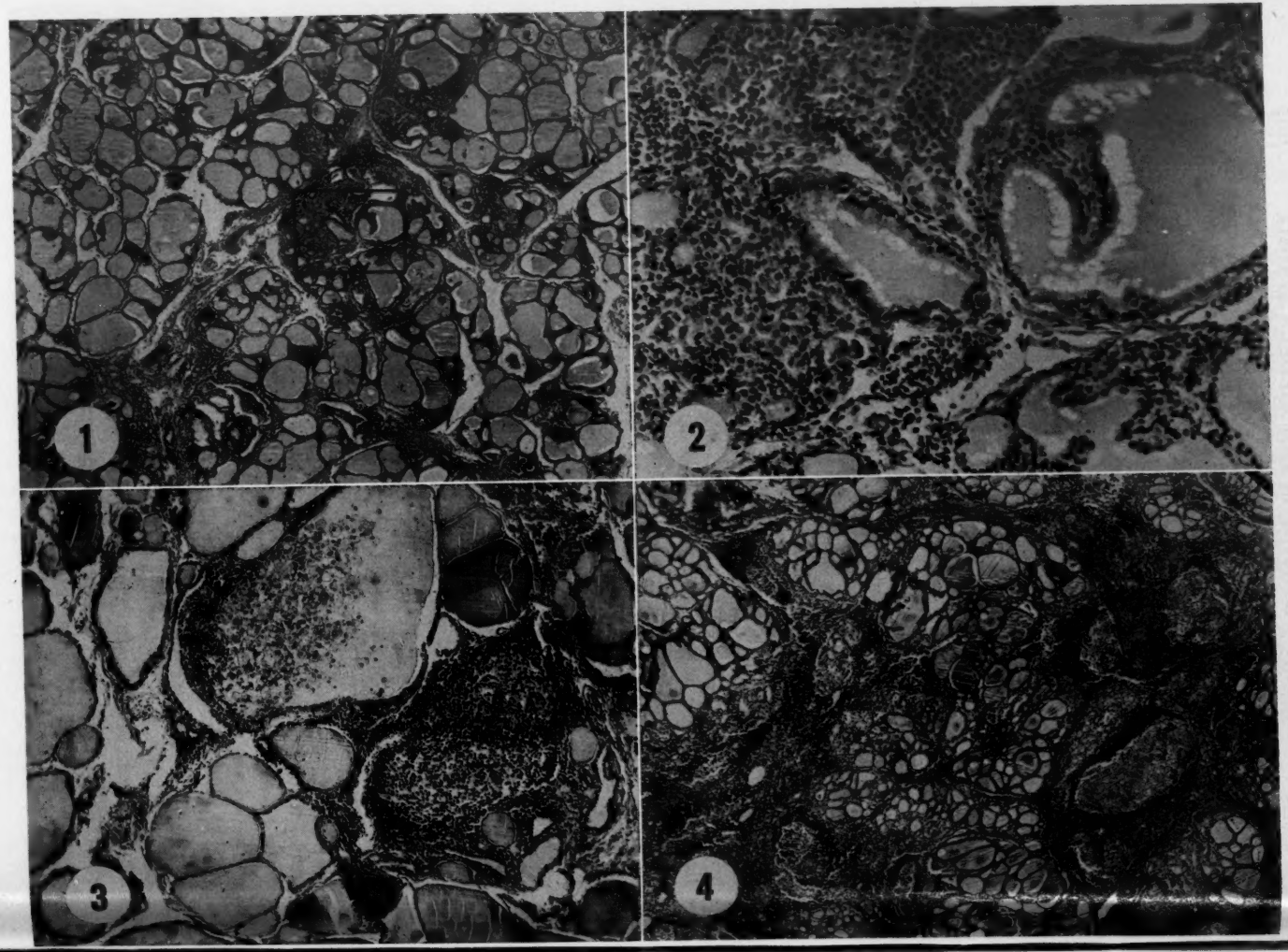
Microscopic: Multiple sections of each specimen generally had a colloid appearance (Fig. 1). Follicles of medium to large size were lined by low columnar epithelium and, in spite of their involution by iodine, their lining cells contained moderate numbers of fine mitochondria. This feature, together with the fact that no fat globules were demonstrable in the cytoplasms, pointed to the fundamentally hyperactive nature of the cells. Here and there papillary infoldings persisted. Lymphoid and plasma-cell infiltrations and lymphoid follicles with germinal centers were notably absent. The involuted parenchyma had failed to evoke infiltration of lymphoid or plasma cells and appeared, therefore, to be devoid of irritative qualities (Fig. 1).

Numerous small disseminated foci of incipient inflammation, on the other hand, contrasted sharply with the wide areas of colloid and uninvolved parenchyma. These focal areas consisted of groups of minute *non-involuted* follicles which were lined by tall hypertrophic epithelial cells containing hyperchromatic nuclei and an abundance of mitochondria. The continued irritative nature of these non-involuted follicles was indicated by the dense collections of lymphoid and plasma cells which had circumscribed them (Fig. 2). Lymphoid follicles with germinal centers were, however, rare. These same non-involuted follicles were for the most part well preserved and hyperfunctioning, as indicated by numerous mitochondria in the cells. Thus they had definitely retained their irritative qualities. Other follicles, however, showed evidences of degeneration, such as atrophy and fragmentation (Fig. 2). Numerous fat globules within the cells attested their initial deterioration. The follicular walls had frequently been invaded by lymphocytes. As a consequence, the lining cells had been freed from their basement membranes and had desquamated into the lumina. They appeared as hydropic epithelial cells in progressive stages of deterioration (Fig. 3). In short, epithelial degeneration due to the lytic action of the round cells was limited entirely to the *hypertrophic non-involuted* follicles within the foci of inflammation. The earliest deposit of fine collagenous and elastic fibers was noticed in these focal areas.

2. *Early phase*

In this group there were 13 female patients whose thyroids showed characteristics of what we have designated as the early phase of chronic thyroiditis. The youngest was a child of 10; the oldest was 49. As in the preceding group, there was no past history of acute or chronic infections. All 13 had had active hyperplastic goiter, with symptoms existing from one to three years—a considerably longer period of clinical activity than in the preceding incipient group. Seven of the patients presented varying degrees of exophthalmos.

All the thyroids were symmetrically enlarged, and moderately firm and hypervascular. However, the increased vascularity and the associated hyperthyroidism in this group, were less marked than in the preceding phase. The metabolic rates were generally moderately elevated and varied between +12 and +31 per cent. The average readings were, therefore, lower than in the preceding group. Eleven of the 13 patients received the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usual preoperative preparation with Lugol's solution. Bilateral partial lobectomy was performed in all instances. Fine adhesions between the thyroid capsules and the prethyroid muscles were frequently encountered.

Macroscopic: The tissues were firm, lobulated, translucent, pale pink and hyperplastic in appearance. There was a perceptible increase in the interlobular fibrous tissue and the capsules were slightly thickened, in contrast to the findings in the preceding group. The parenchyma was fragile and streaks of white fibrous tissue, often appearing as plaques, circumscribed numerous small translucent areas of parenchyma. In 5 of the 13 glands, one or more incidental adenomata were imbedded in the hyperplastic parenchyma.

Microscopic: The inflammation had definitely progressed beyond that in the incipient phase. An average of about one third of the total excised tissue of each gland was involved. The remainder showed reasonably normal parenchyma, the follicles of which were lined by low-columnar and flattened epithelial cells containing reduced numbers of mitochondria. The nuclei were rounded and hyperchromatic. The lumina were filled with homogeneous pink-staining colloid. Surprisingly, all evidences of inflammatory irritation, such as lymphoid and plasma-cell infiltration, lymphoid follicles with germinal centers, and increased fibrosis were totally lacking in the areas of *involuted parenchyma.*

On the other hand, in keeping with the degree of progression of the inflammatory process, the older focal areas showed correspondingly greater variation in size and histologic features. They had obviously been exposed to the damaging effect of the increasing numbers of lymphoid and plasma cells longer than the foci of incipient inflammation

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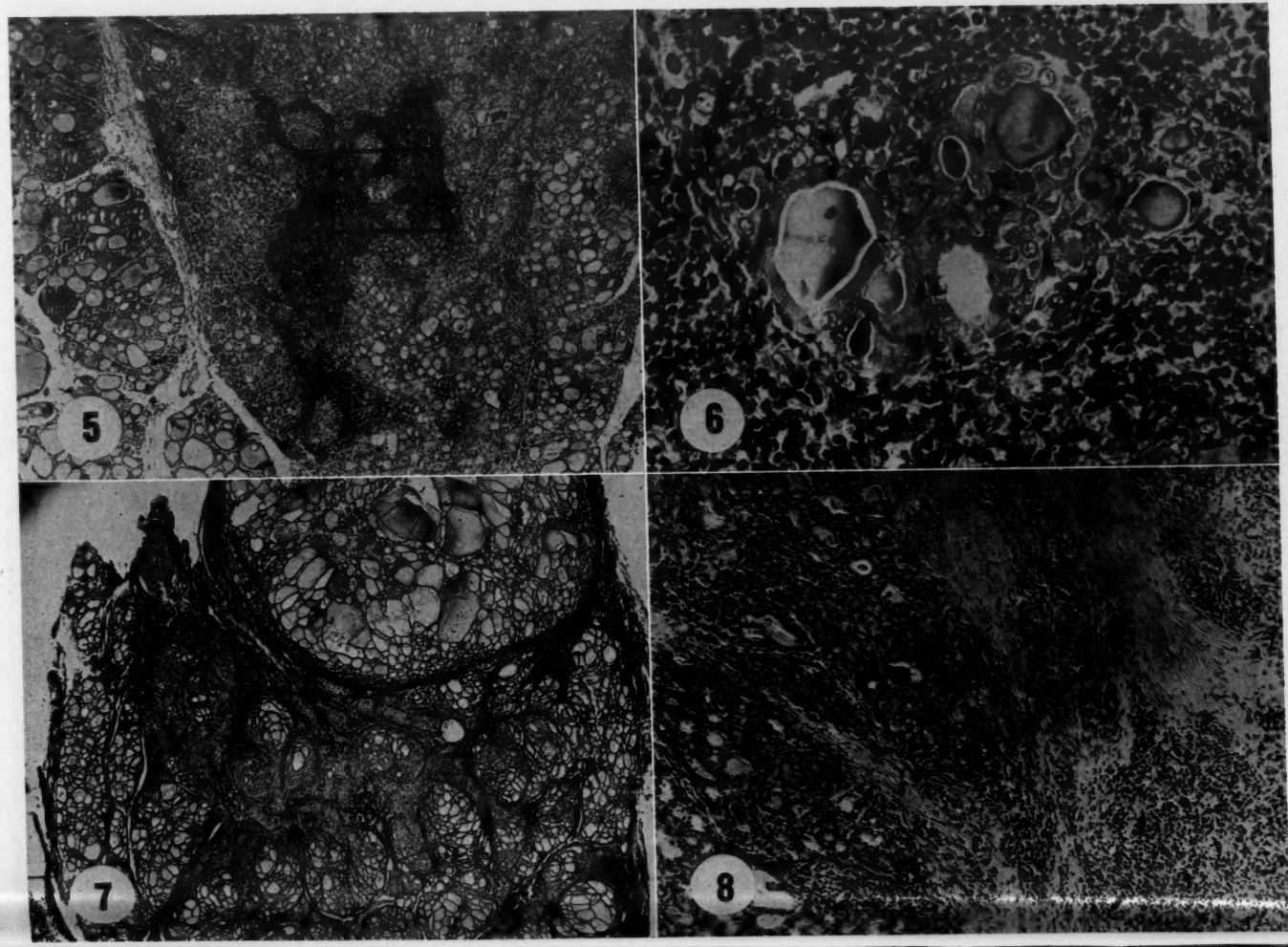
FIG. 1. Incipient chronic thyroiditis. Low-power photomicrograph of a section from the thyroid of a child 9 years old, with toxic diffuse goiter. The basal metabolic rate was +19 per cent. Note the numerous irregular foci of inflammation disseminated in the wide zones of involuted, noninflammatory parenchyma. These foci are composed of irritative, non-involuted hypertrophic follicles circumscribed by collections of round cells. (H. & E. ×30)

FIG. 2. Enlargement of the area outlined in the center of Figure 1. Note in the left half of the section the numerous minute non-involuted follicles, some of which are still well preserved. Their hyperfunction is indicated by their hypertrophic epithelium which contains an abundance of mitochondria. Other follicles, showing atrophy and fragmentation, have succumbed to the lytic action of the infiltrated round cells. Fibrous tissue is rarely increased in this incipient phase. (H. & E. ×200)

FIG. 3. Low-power photomicrograph of a focus of incipient chronic thyroiditis in the hyperplastic thyroid of a girl 15 years of age. In the upper center, the lymphoid cells have infiltrated a segment of the wall of a follicle, the lining cells of which have become detached and have desquamated into the lumen. Parenchymal destruction of this type may be observed in all stages of chronic thyroiditis. (H. & E. ×85)

FIG. 4. Photomicrograph of an area of more intense inflammation, illustrative of early chronic thyroiditis, in the hyperplastic gland of a woman 45 years of age who had suffered acute hyperthyroidism for one year. The pulse was 120 and the post-iodine basal metabolic rate was +17 per cent. Note the confluence of the foci of inflammation, the first appearance of huge lymphoid follicles and the dense concentrations of round cells circumscribing islands of hyperfunctioning parenchyma. Compare with Figure 1. (H. & E. ×30)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(Fig. 4). Accordingly, the residual hypertrophic parenchyma in these same areas manifested greater deterioration, varying from hydrops to atrophy and fragmentation (Figs. 5 and 6). Nevertheless many of the follicular cells, however damaged, had stubbornly resisted complete disintegration. This was manifested by the persistence of an appreciable number of mitochondria. Indeed, many of these isolated and degenerated epithelial cells might easily have escaped recognition if it had not been possible to identify them by the persisting mitochondria. The dense fibrotic stroma of the central areas contained numerous fragments of disrupted plasma cells—the Russell bodies. They represented the end-result of the plasma-cell attack on the hypertrophic epithelium. Furthermore the follicles in these areas showed marked degeneration and desquamation of their lining cells. Broad, interlacing trabeculae enclosed fragments of parenchyma separated by intervening dense collections of round cells and lymphoid follicles with germinal centers.

The smaller, and evidently younger foci were a recapitulation of the findings characteristic of the incipient phase, and again one noted the usual circumscription of groups of small hypertrophic follicles which had not undergone iodine involution. Most of the latter were fairly well preserved. Their hyperfunctioning nature was once again indicated by the numerous mitochondria. Retention of their inherent irritative qualities was again manifested by their chemotactic influence upon the lymphoid and plasma cells. The ratio of lymphoid to plasma cells in the round-cell aggregates was approximately 1 to 3. An occasional artery or vein, occluded by an organized thrombus, was

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FIG. 5. Photomicrograph of an area of early chronic thyroiditis in the hyperplastic thyroid of a woman 52 years of age, who had suffered from hyperthyroidism for two years. The basal metabolic rate was +30 per cent. Note the large irregular aggregate of lymphoid cells isolating islands of parenchyma, and the wide areas of disoriented parenchyma. Within the squared area is an isolated fragment of parenchyma, an enlargement of which is shown in Figure 6. (H. & E. ×30)

FIG. 6. Enlargement of the fragment of hypertrophic parenchyma isolated within the squared area in Figure 5. Beginning atrophy of the columnar epithelium of the larger follicles and disintegration of the disoriented smaller follicles are noted. The nuclei are mostly pyknotic and markedly irregular in size and shape. The lining cells of the intact follicles contain numerous mitochondria, indicating their hyperfunction. (H. & E. ×400)

FIG. 7. Low-power photomicrograph of a simple colloid adenoma, which is sharply demarcated by its thin capsule from the contiguous chronic inflammation in the thyroid lobe. The absence of irritative inflammation in the colloid parenchyma of the adenoma contrasts strikingly with the marked inflammation in the hyperfunctioning thyroid lobe. Fifteen colloid adenomata, occurring in our patients with chronic thyroiditis were, without exception, similarly free of all signs of inflammation. (Weigert and Van Gieson ×12)

FIG. 8. Low-power photomicrograph of an area of intermediate chronic thyroiditis in the hyperplastic thyroid of a 39-year-old patient. Moderate hyperthyroid symptoms had been noted for nine months. The pulse was 94 and the basal metabolic rate, +16 per cent. Note the extensive areas of fibrous replacement and the contiguous disoriented parenchyma. The central zones of fibrotic stroma have assumed the characteristic features of the terminal phase of chronic thyroiditis (Riedel's struma). There is diminished chemotaxis for the round cells in the late phases. (See Figure 9 for H. P. view.) (H. & E. ×85)

observed. Mitoses and polymorphonuclear leukocytes were not noted. There was an early increased deposit of collagenous and elastic fibers in the inflammatory areas. In contrast, no such deposit was noted in the areas of *involuted parenchyma.*

Eight incidental encapsulated adenomata were discovered in the thyroids of 5 of the 13 patients. Three of the adenomata were composed of simple colloid parenchyma (Fig. 7); 1 was of fetal structure; 1 was malignant; and 3 were partially degenerated. Accordingly, an excellent opportunity was afforded to observe the differences in the signs of inflammatory irritation in the two types of parenchyma: 1) the hyperplastic epithelium of the surrounding thyroid lobe, and 2) the hypoplastic parenchyma within the 3 colloid adenomata. Absence of irritating factors in the hypofunctioning epithelium of the adenomata became clearly evident (Fig. 7). The follicles were mostly of large size and were filled with homogeneous colloid. The lining cells were cuboidal or flattened and contained scant numbers of mitochondria with an intermingling of lipoid globules. We found, to our great surprise, that in all 3 instances only the thin capsules of the adenomata separated their colloid parenchyma from the surrounding hyperplastic inflammatory tissue. The colloid parenchyma of the adenomata was completely devoid of evidence of irritation (Fig. 7). We were at a loss to explain how an advancing inflammation could fail to penetrate the virtually microscopic capsule and involve the adenoma. We were forced to conclude that the irritative qualities present in the *hyperfunctioning* epithelium of the thyroid lobe were completely absent in the *hypofunctioning* colloid parenchyma of the adenoma.

3. *Intermediate phase*

There were 14 female patients in this group. Their ages ranged from 28 to 57, and the average was 41 years as compared with 35 in the early phase. There was nothing in their past histories to indicate severe or prolonged bacterial infections. The basal metabolic rates averaged +15 per cent. It is significant that in 5 instances fibrosis had progressed to the point of causing the first mention of pressure symptoms. Three of the patients had had acute hyperthyroidism for eight months; 8 were moderately hyperthyroid at the time of examination and had had symptoms from one to six years; and 2 had been treated for hyperthyroidism for four and six years, respectively. In the latter 2 patients, at the beginning of their complaints the metabolic rates had been +20 per cent and +19 per cent respectively. In one, during the succeeding four years, the basal rate had fallen from +20 to +5 per cent; in the other, during the subsequent five years, the basal rate had declined from +19 to +10 per cent. Thus, in the 2 patients in whom an increased metabolic rate had been noted at the beginning of the illness, there was definite evidence of a decline in the rate associated with continuing thyroiditis over periods of four and six years, respectively. In 13 of the 14 patients it was possible to establish the fact that hyperthyroidism had preceded the progressing chronic thyroiditis by from eight months to five years. In the last of the 14, it was not possible to establish an undoubted history of hyperthyroidism.

Examination: It is noteworthy that in 11 of the 14 patients in this

group, there was no exophthalmos. Mild to moderate prominence of the eyes was noted in 2 instances. The thyroid glands were moderately enlarged. The long-standing inflammation had produced an accentuated lobulation and in a few instances the pre-existing hyperplastic nature of the glands was indicated by persistent increased vascularity. The symptoms of hyperthyroidism and the signs of increased vascularity, however, were distinctly of lesser degree than those noted in the preceding early group.

Bilateral partial lobectomy was performed in all of the 14 cases. In 6, incidental adenomata were found.

Macroscopic: In general, the capsules were moderately thickened, but in several instances they were enormously thickened and hyalinized. Adherent shreds of muscle and fibrous tissue were frequently noted. Both lobes had a pinkish-white sclerotic appearance. The prominent interlacing trabeculae appeared as streaks or plaques enclosing scattered lobules of firm, compact parenchyma. Considerably less of relatively normal translucent parenchyma was apparent than in the preceding early phase. The normal thyroid architecture had been obliterated by the marked fibrosis.

Microscopic: Progression was evident, as compared with the preceding phase (Fig. 8). Extensive areas of inflammation alternated with smaller areas of (colloid) involuted parenchyma. A greater and more extensive deterioration and destruction of glandular epithelium with fibrous replacement was noted. This had resulted in an increased ratio of fibrous tissue to uninvolved gland.

Large areas of normally involuted parenchyma still persisted. The follicles were lined with hypertrophic epithelial cells containing hyperchromatic nuclei and many mitochondria, indicating once again their hyperfunctioning nature. The lumina were partially filled with desquamated epithelial cells intermingled with moderate numbers of round cells. The parenchymal lobulation was accentuated by heavy interlobular trabeculae. Small collections of cells, predominantly lymphoid, were occasionally seen but lymph follicles with germinal centers were entirely absent (Fig. 8).

Possibly two thirds to three fourths of the total gland substance had now been involved in changes of great histologic variability. The lesion had the characteristic features of the incipient and early phases, with added manifestations of greater and more advanced progression. Areas of earliest inflammation appeared at the peripheries of the larger and older inflammatory areas. The dense central zones were almost devoid of any surviving parenchyma (Fig. 8) and had begun to assume many of the histologic features of Riedel's struma. Occasional islands of non-involuted parenchyma, isolated in the stroma of the central older inflammatory areas, showed varying degrees of damage. Mitoses, which might be interpreted as indicative of compensatory epithelium, were not noted. Persisting islands, which consisted of fragmented epithelial cells with shrunken pyknotic nuclei and scant atrophic cytoplasm, could readily be identified. They consisted of residual hyperplastic epithelium and hence retained fair numbers of mitochondria (Fig. 9). They were hardly viable and consequently had lost much of their irritative nature, as indicated by their diminished chemotaxis for the round cells. Numerous lymphocytic collections of varying size were seen in the less dense stroma. However, lymph follicles with germinal centers were rare. Plasma and lymphoid cells appeared in definitely reduced numbers as compared with their increased numbers in the earlier phases. Numerous fragments of disintegrated plasma cells—the Russell bodies—which had succumbed in their attack against the hyperfunctioning epithelium,

were recognized here and there in the stroma. The lining cells of the hypertrophic thyroid follicles in the involved parenchyma had desquamated because of the lytic action of the circumscribing round cells. They had partially filled the lumina and there had formed the pseudo-giant cells. Diminished numbers of mitochondria and a profusion of lipoid droplets further confirmed the deterioration of these cells. Elastic fibers had increased in direct ratio to the increased fibrosis, whereas in areas of normally involuted parenchyma they were virtually absent. Many veins, occluded by organized thrombi, were easily identified by the persistence of elastic fibers derived from their adventitiae. Thrombosis of vessels, with resultant ischemia and diminished nutrition, was an additional factor in epithelial degeneration.

Fourteen adenomata, varying from 1 to 3 centimeters in diameter, occurred in the thyroids of 6 of the patients in this group. Ten of them were of the simple colloid type. The findings were exactly the same as in the 8 colloid adenomata described in the early phase. Again, the hypofunctioning colloid parenchyma of the adenomata was strikingly devoid of all signs of inflammation.

4. *Terminal phase*

The 12 patients in this terminal phase of chronic thyroiditis, identical with Riedel's struma, were females and were appreciably older than those in the intermediate group. Their ages ranged from 36 to 59. A history of infections could not be elicited. Upon careful inquiry regarding possible antecedent hyperthyroidism, the following information was obtained. In 1 of the patients the evidence was presumptive, since she had been treated with Lugol's solution for a "thyroid condition" fifteen years previously. Three patients had nervous manifestations characteristic of chronic hyperthyroidism over a period of five to six years. Two complained of acute pain due to a recent typical subacute thyroiditis with symptoms of mild to moderate hyperthyroidism (subsequent partial thyroidectomy revealed typical Riedel's struma). Three had noted symptoms of preceding hyperthyroidism for periods of one to three years. One patient had been treated for hyperthyroidism by a well-known specialist in thyroid diseases over a period of four years; then followed an interval of eleven years, during which the history was uncertain. One complained of symptoms of what was presumably chronic hyperthyroidism (supported by the fact that she had auricular fibrillation). Another patient gave a history of three recurring episodes of hyperthyroidism over a period of twenty years, and her heart presented findings of impending auricular fibrillation. Thus we have reason to believe that the terminal phase of chronic thyroiditis in 11 of the 12 cases had been preceded by chronic hyperthyroidism extending over periods ranging from a few to many years. In 1 instance only, the evidence was merely presumptive.

Mild symptoms of hyperthyroidism were observed in a few instances. Although symptoms of moderate pressure were first noted by several patients in the intermediate group, symptoms of increasing pressure were experienced by most of the patients in this terminal group.

Four patients had moderate hyperthyroidism and 3 presented findings of mild to moderate myxedema. The differences in symptomatology could be attributed to varying degrees of destruction of the parenchyma and to the secretory potential of the surviving epithelium. Singularly, exophthalmos was noted in only 1 of the 12 patients.

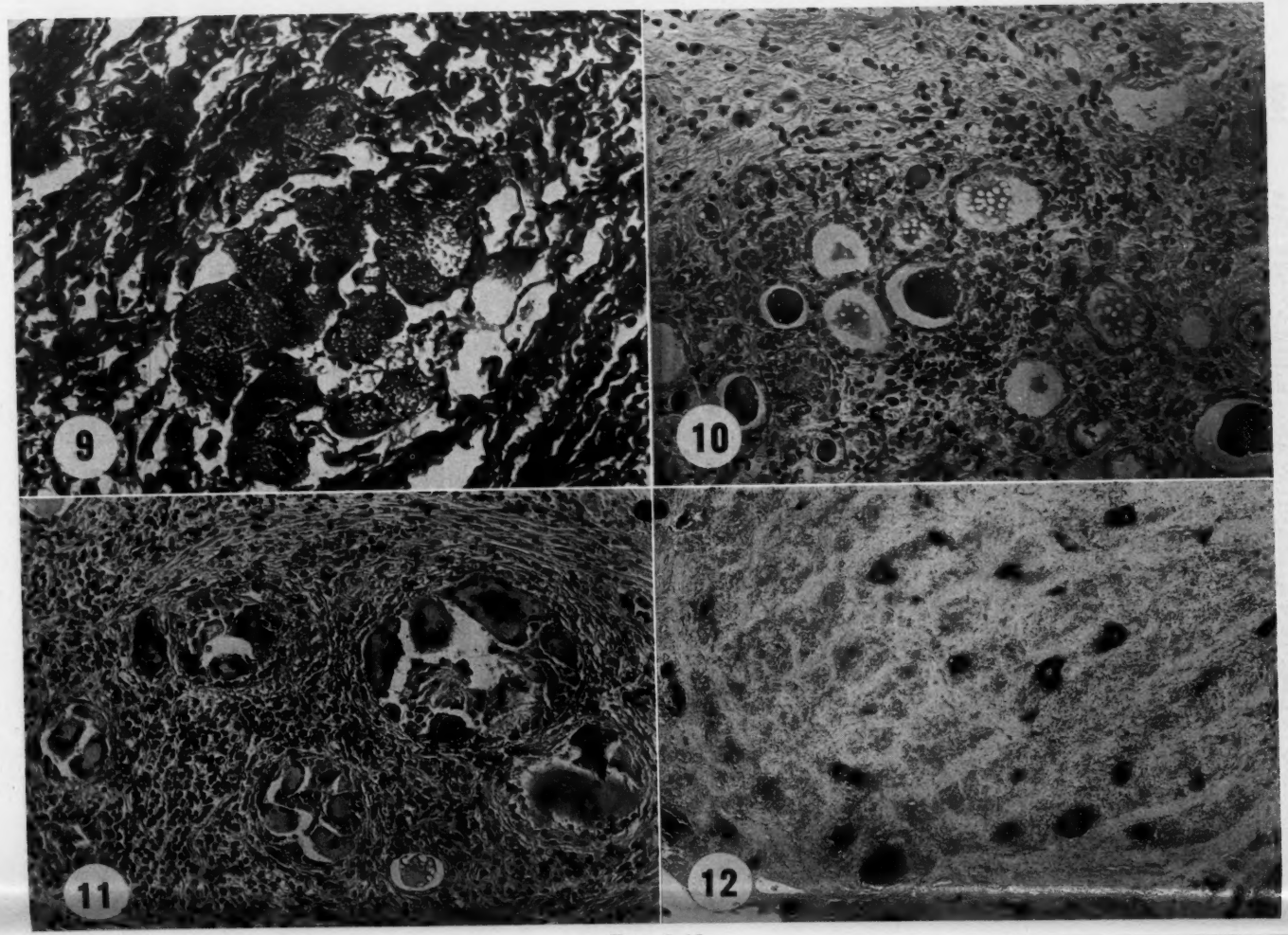
The basal metabolic rates tended definitely to be lower than in the intermediate-phase group. In 5 of the 12 patients the rates ranged downward from +12 to +2 per cent. In 3 of the most advanced cases, in which surviving remnants of thyroid parenchyma were difficult to find, the metabolic rates were −11, −21 and −22 per cent respectively. These were the patients who had mild to moderate myxedema. In 1 of 2 patients who had only recently experienced symptoms of subacute thyroiditis, the basal metabolic rates were reported as elevated. In the 2 remaining instances the rates had not been determined. Thus in 10 of the 12 patients the basal metabolic rates had, over the years, declined to levels considerably below those observed in the preceding intermediate phase.

The principal physical features of the thyroids were secondary to far-advanced fibrous replacement. The moderately enlarged glands were approximately normal in contour with well defined lobulation and an unusually firm consistency. There were no signs of increased vascularity, in sharp contrast with the diminished signs of increased vascularity frequently observed in the intermediate phase.

Macroscopic: The thickened capsules commonly showed adherent shreds of muscle and fibrous tissue. The cut surfaces of the glands were pinkish-white in color and revealed firm, compact areas of yellowish-white atrophic parenchyma. Fibrous replacement had occurred approximately equally throughout both lobes. The stroma was characterized by broad, anastomosing bands of hyalinized collagenous tissue, which enclosed insignificant amounts of granular, pinkish or pale-yellow atrophic parenchyma in its well defined spaces. Normal thyroid tissue was entirely obliterated, in contradistinction to its preservation in fairly large areas in the preceding phase. Incidentally, four pyramidal lobes showed extreme fibrosis and complete absence of normal-appearing parenchyma.

Microscopic: Extensive damage of the greatly reduced epithelium and generalized fibrosis were strikingly apparent in all the specimens (Fig. 10). Mere remnants of colloid parenchyma, strikingly free of infiltration of plasma cells, lymphocytes and fibrous tissue, alternated with fragments of degenerating hypertrophic parenchyma. All the glands exhibited the histo-pathologic criteria of Riedel's struma. Whereas these same criteria were applicable to only limited areas of advanced inflammation in the preceding phase, they were now applicable to the total gland substance in this terminal stage. Lymph follicles with germinal centers were rarely noted. Degeneration of surviving colloid remnants, incarcerated in the stroma, was apparent in the clumping of the mitochondria and the presence of numerous lipoid bodies in the epithelial cells. The degeneration appeared to have resulted from the strangling effects of the surrounding fibrosis rather than from primary lytic action of plasma and lymphoid cells, which were no longer present.

The most striking feature was the enormous and generalized increase in the relatively



FIGS. 9-12

acellular stroma. Broad bands of hyalinized collagenous tissue subdivided the scant amounts of persisting parenchyma into isolated islands of varying size. Surprisingly, many of the follicles composing these remnants had retained evidences of former hyperfunction. The columnar epithelium retained its hyperchromatic nuclei and numerous mitochondria. Furthermore, these cells preserved their chemotactic influence on the lymphoid cells which encircled them. There were still other small persisting fragments which exhibited terminal disintegration. Eventually only the shunken pyknotic nuclei, with attached fragments of cytoplasm, remained. These disintegrating fragments contained a minimum of faintly stained mitochondria intermingled with numerous black lipoid globules. The lipoid bodies occurred in indirect ratio to the diminished numbers of mitochondria.

Numerous minute hypertrophic follicles within the lymphoid conglomerates were observed. They had not, as yet, completely succumbed; they had fused into syncytia of vacuolated protoplasm containing shrunken nuclei, numerous fat droplets and deteriorating mitochondria. The degenerated epithelium of the syncytia had exerted a greatly diminished chemotaxis upon the round cells. The larger collections of round cells and lymphoid follicles with germinal centers, observed in preceding phases, were strikingly infrequent. Here and there numerous Russell bodies were recognized.

A further striking feature, typical of Riedel's struma, was noted in the desquamation of the follicular cells into the lumina of many of the larger follicles (Fig. 11). These desquamated cells were generally rich in mitochondria. They had frequently fused into masses of multinucleated protoplasm—the pseudo-giant cells. Eventually fibroblasts converted the conglomerate of the pseudo-giant cells, remnants of colloid, rare plasma cells and lymphocytes into a dense obliterating fibrosis in which fragments of the pseudo-giant cells could still be recognized. Groups of surviving epithelial cells, which

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FIG. 9. High-power photomicrograph of a remnant of hypertrophic epithelium incarcerated in the fibrotic stroma of Figure 8. The cells are still rich in mitochondria and resemble the cells of antecedent hyperplastic goiter. They are not compensatory epithelium. Such incarcerated remnants of parenchyma are commonly seen in the stroma of advanced chronic thyroiditis. (Mallory ×450)

FIG. 10. Moderate enlargement of a small fragment of surviving parenchyma, isolated in the hyalinized stroma of the terminal phase. Note the atrophy and degeneration of the hypertrophic follicles. The plasma and lymphoid cells are few in this final phase. The amount of surviving epithelium and its secretory potential were indicated by a basal metabolic rate of +12 per cent. The patient, a woman of 52 years of age, had suffered from chronic hyperthyroidism for fifteen years; in recent years she had had symptoms of local pressure. (Zenker-Gram stain ×200)

FIG. 11. Photomicrograph of an area of terminal chronic thyroiditis (Riedel), illustrating the disintegration of the hypertrophic follicles with desquamation of the follicular epithelium and the formation of pseudo-giant cells. Notwithstanding this circumstance, numerous mitochondria are still demonstrable in the giant cells, resembling in this respect the hypertrophic follicular cells from which they were derived. The giant cells eventually disintegrate and disappear in the fibrosis of the follicle. (H. & E. ×200)

FIG. 12. Low-power photomicrograph of far advanced chronic thyroiditis, illustrating the extreme fibrous replacement of the thyroid epithelium. There is consequently a lack of chemotaxis for lymphoid and plasma cells. The patient was 54 years of age and had myxedema and local symptoms of pressure. The basal metabolic rate was −22 per cent. (H. & E. ×12)

were incarcerated in the stroma and which have often been misinterpreted as compensatory in nature, failed to show true mitoses and an increase in mitochondria. Both of these findings should be observed in compensatory regeneration. Polymorphonuclear leukocytes were extremely rare.

Our most extreme example of advanced chronic thyroiditis (Riedel's struma) was well illustrated in one of our myxedematous patients (Fig. 12). The basal metabolic rate was −22 per cent. The entire thyroid had been converted into a hyalinized stroma in which there was a striking absence of even approximately normal-appearing thyroid tissue. However, minute groups of epithelial cells, disseminated in the stroma, were identified as surviving cells of formerly hyperfunctioning parenchyma, on the basis of their increased content of mitochondria. Plasma cells, no longer required, were also almost completely absent.

Incidentally, 9 adenomata measuring 1.25 to 4.0 centimeters in diameter were present in the thyroids of 5 of the 12 cases. Two of these were composed wholly of colloid parenchyma, and the remaining 7 showed varying types of degeneration. Singularly, as in the case of the purely colloid adenomata occurring in the early and intermediate phases, 2 colloid adenomas of this group also failed to show the faintest evidence of inflammation.

DISCUSSION

The etiology of chronic thyroiditis and its terminal phase of Riedel's struma is unknown and the pathogenesis is equally obscure. An extensive review of the literature has revealed that a multitude of theories and explanations, largely unsupported by adequate clinical, histologic or experimental evidence, have been held. Our studies do not support the assumption, frequently expressed, that infection plays an important role. In fact the patients in our studies had been unusually free from acute or chronic infections.

Simmonds (45) pointed out that collections of lymphocytes occurred in 75 per cent of the thyroids in Graves' disease. Many authors have reflected on the significance of such lymphoid aggregates, commonly found in both Graves' disease and chronic thyroiditis. It was felt that if one could account for the infiltration of lymphoid and plasma cells, an etiologic interrelationship of lymphoid tissue, thyroid hyperplasia and chronic thyroiditis might be discovered. We cannot agree with Levitt (52), who felt "that the lymphoid cells surround and appear to replace hypoplastic atrophying epithelial cells." We found, on the contrary, that the greatest predominance of lymphoid tissue was in the early phases of the inflammation, long before extensive epithelial atrophy and disintegration had occurred. It appeared, accordingly, that the lymphoid infiltration was the primary cause, and not the result of the atrophy and final disintegration.

Many investigators have searched so long for extrinsic causes that they have tended to overlook possible intrinsic etiologic factors. Our studies indicated that the reaction of lymphoid tissue and plasma cells is in the nature of a chemotactic response to inherent toxic factors residing in the hyperfunctioning epithelium per se. Furthermore, it seemed that, in con-

trast with the normal condition, the hyperplastic follicular cells act as irritants and provoke a protective response on the part of the host. Interestingly, this response had several points of resemblance to that seen in certain types of scirrhous carcinoma.

Our views regarding etiology gained additional significant support from a critical study of 15 *colloid* adenomata observed incidentally in our cases of hyperplastic goiter. In contrast to the total absence of any evidence of irritation in the colloid parenchyma of the adenomata, the surrounding hyperplastic parenchyma invariably showed varying degrees of inflammation (Fig. 7). The conclusion seemed inescapable that these irritative qualities were absent in the hypofunctioning colloid parenchyma of the circumscribed adenomata. Otherwise one would be at a loss to explain the failure of a generalized inflammation to penetrate and involve the substance of the adenoma, especially when the capsule was generally almost microscopic in thinness. The fate of the hyperplastic epithelium assumed great significance in our pursuit of the progress of the lesion to its terminal stage. Hence the continued recognition of the gradually disappearing hyperactive epithelium became of primary importance, especially when the histologic characteristics had been altered by both the administration of iodine and by the inflammation itself. Particularly in the later fibrotic stages, the degree of hyperactivity could not be based on the usual conventional criteria, such as papillary infolding and the increase in size and height of the acinar cells. In this situation the behavior of the mitochondria became most helpful. They were found to be very resistant to the lytic action of the round cells. Moreover, by their persisting presence, we were able to recognize slowly deteriorating but previously hyperactive cells that might otherwise have escaped detection (Fig. 9).

We are in general agreement with Levitt (52), who described six phases, from epithelial hyperplasia to the terminal stage of fibrosis typical of Riedel's struma. The phases were naturally not sharply delineated but progressed gradually from one to the next. The limited areas of fibrosis in one phase became the predominant feature of the next. Thus, for example, the restricted areas of advanced inflammation of the intermediate phase had, to a considerable extent, assumed the histologic appearance of Riedel's struma.

Chronic thyroiditis may extend over long periods—even two or three decades—and terminate in virtually complete destruction of the epithelium. However, it should be stressed that each stage does not necessarily advance to the next. The steady progress may be halted at any time that the irritating epitehlium has been destroyed in sufficient amounts to cause a retrogression toward normal of the residual parenchyma, after

the fashion of the thyroid remnants following partial resection. Obviously, if all early cases were to continue unchecked, Riedel's struma would be encountered much more frequently. Incidentally, any residual parenchyma which had stubbornly resisted dissolution in the earlier stages might finally be destroyed by the ischemia secondary to fibrosis and the commonly observed venous thromboses. However, complete fibrous replacement need not necessarily always occur in chronic thyroiditis any more than it does in the islets of Langerhans in diabetes or in the kidneys in chronic nephritis. Finally chronic thyroiditis probably represents a benign healing process which may, however, at times over-compensate and then result in hypothyroidism and even myxedema.

SUMMARY

Chronic thyroiditis is an inflammatory lesion which, from its incipiency to its terminal stage of generalized fibrosis, runs a protracted course. It is characterized by great variability in its histologic features. For purposes of description, it was convenient, albeit somewhat arbitrary, to subdivide its course into four phases: incipient, early, intermediate and terminal. By tracing the lesion retrogressively, we identified the incipient basic pattern which consisted of round-cell circumscription of groups of minute, *non-involuted, hypertrophic* follicles disseminated in the parenchyma of toxic hyperplastic goiter (Figs. 5 and 6). Incidentally, Boyden, Coller and Bugher (9) noted that the small hypertrophic follicles were almost invariably incorporated in, or adjacent to, collections of lymphoid and plasma cells. As a result of the lytic action of the circumscribing lymphoid and plasma cells, these *non-involuted* hypertrophic follicles were caused to deteriorate and eventually disintegrate. This basic pattern was repeated over and over in succeeding phases. Accordingly, when the later phases were compared with the earlier ones, it was readily recognized that the pathologic changes were matters of degree—quantitative and not qualitative. That the *non-involuted hypertrophic follicles* within the focal areas of inflammation were clearly hyperfunctioning was indicated by their rich content of mitochondria. Moreover they were evidently irritative, as judged by their chemotactic influences upon the lymphoid and plasma cells. It appeared that the epithelial cells of the hyperplastic gland were in different phases of secretory activity and sensitivity to iodine at any given moment. Consequently they varied correspondingly in their potentiality for provoking irritative responses. From time to time, new foci of hypertrophic epithelium appeared, following which the inflammatory cycle was repeated. After numerous repetitions of this cycle, the bulk of the hypertrophic parenchyma was correspondingly reduced.

The wide areas of *colloid, involuted follicles* were by contrast strikingly

devoid of lymphoid and plasma-cell infiltration. The temporarily curtailed function of these colloid follicles was indicated by their sharply reduced content of mitochondria, whereas mitochondria occurred in abundance in the non-involuted follicles. This striking difference in the responses of the lymphoid and plasma cells to the involuted and the non-involuted phases of the epithelium, respectively, was a remarkably constant finding throughout the subsequent course of the lesion. The difference appeared to be determined by the irritative nature of the *non-involuted follicles*, whereas the parenchyma, which had shown the capability of *involution* by iodine, was surprisingly devoid of inflammatory cells. Deposits of fine collagenic and elastic fibers in the inflammatory foci of the incipient phase were barely noticeable.

As the lesion advanced, it was characterized by features which we have conveniently designated as the early phase. In this group the thyroid glands were typically hyperplastic and histologically presented a more intense inflammation. More numerous focal areas of hyperfunctioning epithelium were involved. They were characterized by larger and denser concentrations of lymphoid and plasma cells. Lymph follicles with germinal centers began to make their first appearance (Fig. 4). Moreover, because of the more intense inflammatory response on the part of the lymphoid tissue and the considerably longer time that it had been active, correspondingly greater destruction of hypertrophic epithelium was noted.

The parenchyma of the glands in the first two phases had, however, not as yet been destroyed in amounts sufficient to produce a significant diminution in the total secretory potential. Continued progression resulted in increased production of hyalinized stroma in the central zones of inflammation. Approximately one third of the total parenchyma showed inflammatory involvement; the balance, consisting of colloid involuted parenchyma, was by comparison free of all evidence of inflammation.

By gradual transition and continued progress the inflammatory lesion reached the next or intermediate phase. Approximately two thirds of the parenchyma had become involved, as against possibly one third in the preceding phase. The older central areas of hyalinized stroma began to assume, more and more, the appearance of Riedel's struma. Groups of surviving incarcerated epithelial cells showed an increased content of persisting, though appreciably deteriorated, mitochondria (Fig. 9), indicating their derivation from antecedent active hyperplasia cells which they still closely resembled. The prominent chromatin figures within these cells were interpreted by Eisen (40) and others (31, 32) as mitoses and regarded as isolated remnants of compensatory epithelium. We cannot agree with this view, for had they been of a compensatory nature, they would hardly have fallen victims of deterioration. Furthermore they were

also observed in the limited areas of fibrosis of the earlier phases, when there obviously was no thyroid deficiency requiring compensatory secretion. Finally this supposed compensatory epithelium failed to counteract the downward progression of the hypothyroidism in the later stages. Diminished infiltration of lymphoid and plasma cells was clearly evident, because of the reduced amount of residual parenchyma still capable of exercising chemotaxis for inflammatory cells. Moreover the ratio of lymphocytes to plasma cells had now decreased, whereas in the previous phase the reverse obtained. Also lymph follicles with germinal centers were now rare.

As in the preceding phases, an interesting mode of destruction of the larger as well as the smaller follicles was observed (Figs. 3 and 11). The follicular cells had become detached from their basement membranes by the lytic action of the circumscribing round cells and had accordingly desquamated. Subsequently they had coalesced into epithelial conglomerates which formerly were mistakenly interpreted as true phagocytic giant cells (14:16) but which were later demonstrated to be "pseudo-giant" cells without phagocytic properties (Goetsch (18)).

Chronic hyperthyroidism had definitely preceded the terminal phase of chronic thyroiditis in some cases by as much as fifteen years. With due allowance for the inability of the occasional patient to recollect symptoms that had been present many years before she first presented herself, the histories of the 12 patients in the terminal phase afforded considerable additional support to our view that thyroid hyperplasia does, in fact, precede chronic thyroiditis, particularly its terminal phase of Riedel's struma.

Finally the terminal phase evolved by further transition and ended in virtually complete destruction of the thyroid epithelium, which was replaced by fibrosis and even compact hyalinized stroma typical in every respect of Riedel's struma. The glands were unusually firm, "iron hard" and had caused symptoms of varying degrees of pressure in most of the patients. With the disappearance of the irritative hyperfunctioning epithelium there was a striking lack of chemotaxis for the plasma and lymphoid cells, which had been noted to be relatively numerous in the previous phases. They now appeared as mere small aggregates clustered about the smaller arteries (Fig. 12).

Polymorphonuclear leukocytes were strikingly rare in all phases, and mitoses were almost never observed. Accompanying the progressive diminution and destruction of the epithelium there was a parallel proportionate increase both in the replacement fibrosis and the elastic tissue.

Our views on etiology received further significant support from the vari-

able behavior of the lymph tissue and plasma cells. In the earlier phases, in which the hyperfunctioning epithelium was most abundant, there was a correspondingly profuse and frequently dense infiltration of round cells. In the later phases, in which the provocative epithelial cells had been largely destroyed, a parallel marked diminution in the amount of lymphoid tissue, and particularly of plasma cells, was noted.

The symptoms throughout reflected the lessening amounts and the secretory potential of the parenchyma. The time required for the development of Riedel's stuma from early thyroiditis is necessarily protracted and variable. It embraces 1) an initial period of hyperthyroidism followed by 2) an amelioration of the symptomatology, and 3) a gradual transition toward a state of hypothyroidism. When, in the occasional case, the advancing lesion is not halted, it proceeds to 4) its termination in Riedel's struma. The basal metabolic rates also closely parallel the decline in the symptomatology and in our cases ranged from a high of +42 per cent in the incipient phase, to a low of −22 per cent in the terminal phase. Incidentally, there was a concomitant diminution in the vascularity of the glands.

It was interesting to note how the average ages of the patients in the four groups increased in direct proportion to the duration of the lesion. The averages were 26, 35, 41 and 48 years respectively. Notably, there were wide variations in the incipient-phase group, indicating that the inflammation depended upon the time of onset of the hyperthyroidism rather than upon the age of the patient. Generally speaking, the incipient inflammation occurred in the younger individuals. There were actually 4 children under 12 years of age in this group.

With few exceptions, all of our patients were females. Any attempt to explain the definitely increased frequency in the female sex must await satisfactory explanation of the greater frequency of toxic diffuse goiter in the female.

Singularly the exophthalmos was noted most frequently in the earlier phase during which the thyroid showed a marked potentiality for involution following the administration of iodine. This contrasted sharply with the infrequency of exophthalmos in the later phases. Thus it occurred only once among the 12 patients with terminal thyroiditis (Riedel's struma). The tendency of exophthalmos to diminish in frequency as more and more of the gland is destroyed was similarly pointed out by Levitt (52).

Although we are conscious of many problems remaining unanswered, our studies have given us a better understanding of the hitherto obscure etiology and pathogenesis of chronic nonspecific thyroiditis which occasionally progresses to Riedel's struma.

CONCLUSIONS

1. A comprehensive review of the extensive literature indicates that the etiology of chronic nonspecific thyroiditis is still unknown.

2. Critical studies over many years warrant the concept that chronic, nonspecific thyroiditis is an inflammatory response to etiologic irritative factors residing in the hyperfunctioning epithelium of primary hyperplastic (toxic diffuse) goiter.

3. In its incipiency, this response is characterized by circumscription, isolation and progressive destruction of numerous irritative foci of hyperfunctioning parenchyma by lymphoid and plasma cells.

4. The incipient qualitative pattern continues through several successive stages and develops into a massive quantitative one. By gradual transition over protracted periods the inflammation advances to a terminal stage in which there is virtually complete destruction of the thyroid parenchyma and replacement by fibrous tissue.

5. The lesion need not always advance to the terminal stage. The pathologic process may be halted at any time during its course. Otherwise the terminal stage would occur much more frequently.

6. Our findings appear to support the view that Riedel's struma is the end-result of successive phases of chronic nonspecific thyroiditis.

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Editorial

METHODS FOR ASSESSMENT OF ADRENOCORTICAL FUNCTION

PRESENT methods for the determination of blood and urinary steroids have been useful in the diagnosis of adrenal disorders, but experience has shown that their usefulness is limited. Although the onus for this limitation usually falls on the methods, pathophysiologic variations are such that it would be too much to expect that even a technically perfect method would lead always to the correct diagnosis. It is the purpose of this discussion to consider some available methods and recent developments that render them more useful for clinical purposes, particularly in the diagnosis of adrenal insufficiency.

In Addison's disease, values for the urinary 17-ketosteroids usually are well below the normal limit, but values of a similar order of magnitude may be obtained in various disorders when other evidence indicates that adrenocortical function is not decreased. Normal levels of urinary 17-ketosteroids may help to rule out the presence of this disease, but occasionally normal levels are found in the face of clinically evident Addison's disease. The 17-ketosteroid values are most helpful in the diagnosis of adrenocortical tumors and the adrenogenital syndrome.

The formaldehydogenic method (1) for the determination of the level of urinary corticosteroids extractable at pH 1 has proved to be useful in the diagnosis of Cushing's syndrome in about 90 per cent of cases, but the values in cases of Addison's disease and in cases of pituitary failure often overlap those of normal individuals. Also, substances in urinary extracts may interfere with this procedure.

The method of Reddy and associates (2) for the assay of conjugated urinary steroids that have a side chain like that of cortisone (17,21-dihydroxy-20-ketosteroids, often termed 17-hydroxycorticoids) depends on the reaction of steroid and phenylhydrazine in a strong solution of sulfuric acid (Porter-Silber reaction (3)). Unfortunately, the action of strong sulfuric acid alone on some organic substances is such that a large blank value is obtained with urinary extracts that contain the conjugated steroids. As a result the net light absorption of the steroid-phenylhydrazine product is only the small difference between two large values. Indeed, there have been reports that the reading of the extract plus sulfuric acid (the blank) sometimes may be greater than that of the extract plus the

complete reagent. The original method has been modified in several respects in order to reduce the blank value, but it has not been reduced sufficiently to permit confidence in the absolute values obtained. This uncertainty is reflected in discrepant normal ranges given by Liddle and associates (4) and by Jenkins and associates (5). The former group obtained values ranging from 5 to 16 mg. with a mean of 10 mg. per twenty-four hours, whereas the latter group obtained values of 1 to 10 mg. and a mean of 5.2 mg. per twenty-four hours. According to Liddle and associates, in cases of adrenal disease there is some overlapping of data at both extremes of the normal range, patients with Addison's disease giving values of 0 to 5 mg. and patients with Cushing's syndrome, values of 15 to 70 mg. per twenty-four hours.

Nelson and Samuels (6) have developed a method for assay of blood corticosteroids which involves chromatographic isolation of hydrocortisone (also including cortisone and Reichstein's substance S, if present) and application of the Porter-Silber reaction. The hydrocortisone level is considered to be a good index of adrenocortical function, since hydrocortisone appears to be the hormone secreted in largest amounts by the human adrenal. This method has been used rather widely for investigative purposes, but the chromatography is time-consuming and has been a source of some difficulty. More recently Silber and Porter (7) have published a much simpler method for the determination of plasma 17,21-dihydroxy-20-ketosteroids. This method involves extraction of plasma with chloroform, washing the extract with alkali, and extraction of the steroid from the chloroform solution with the sulfuric acid-phenylhydrazine reagent. The procedure appears to be specific and gives results similar to those of the Nelson-Samuels method.

The plasma levels are subject to diurnal variations and to environmental factors. It is evident that single determinations may give little information of importance and may be entirely misleading. For example, Eik-Nes and associates (8) found plasma levels that were well within the limits of normal values in 3 of 6 patients with Addison's disease. Among 12 cases of Cushing's syndrome I have encountered 4 with 8 A.M. values and diurnal variations well within the normal range.

It is clear that none of the methods for the determination of urinary or blood steroids is able to furnish clean-cut evidence of adrenocortical hypofunction or hyperfunction in all cases. However, the use of corticotropin in conjunction with these methods may furnish unequivocal evidence of adrenocortical insufficiency when the methods themselves have failed to do so. Unfortunately, corticotropin does not always cause a significant increase in the excretion of 17-ketosteroids by normal individuals. Thus, Jenkins and co-workers (5) found an increase of less than 2 mg. per

twenty-four hours in 14 per cent of control cases when corticotropin was given by intravenous infusion during eight hours or by injection of corticotropin gel. This change was less than in some cases of Addison's disease. Others have made similar observations.

The response of the conjugated 17-hydroxycorticoid excretion to the administration of corticotropin was much more favorable. The increase was less than 5 mg. (but not less than 4.5 mg.) per twenty-four hours in only 5 per cent of the control cases, whereas the maximal increase in 10 cases of Addison's disease was 1.9 mg. In spite of the objections raised, the method of Reddy and associates (2) appears to be adequate to distinguish significant differences in the amounts of 17-hydroxycorticoids excreted before and after administration of corticotropin. Determination of the formaldehydogenic steroids extractable from urine at pH 1 is equally useful in conjunction with the administration of corticotropin.

Eik-Nes and associates (8) combined prolonged intravenous infusion of corticotropin with measurement of blood levels of corticosteroids. In the case of the 3 patients with Addison's disease who showed normal blood levels, there was little or no increase in the blood levels during an eight-hour intravenous infusion of corticotropin. The results suggest that these patients had a small supply of adrenocortical hormones which could not be increased in response to physiologic demands.

The chemical methods themselves are subject to improvement, particularly with respect to specificity, but improvement in these methods or substitution of better ones still leaves the problem of individual variations. Present methods, when corticotropin is used in conjunction, appear to be reasonably adequate for the diagnosis of adrenal insufficiency. The picture is not quite as clear with respect to the differentiation of hyperfunctioning lesions of the adrenal cortex. However, the methods of assay are adequate to show increased (adenoma or hyperplasia in Cushing's syndrome) or unchanged (carcinoma) steroid production after administration of corticotropin, or the suppression of 17-ketosteroid excretion during administration of cortisone to patients with the hyperplasia associated with the adrenogenital syndrome (9, 10).

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(For program, see July issue)

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.

2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

INTER-SOCIETY CYTOLOGY COUNCIL

The third Annual Meeting will be held in the Statler Hotel, Cleveland, Ohio, Friday and Saturday, November 11 and 12, 1955. Everyone interested in cytology is invited to attend.

Morning and afternoon sessions will consist of sixteen formal papers and nine round table discussions of cytological problems. There will also be a symposium on "Problems of Early Endometrial Cancer."

The annual banquet will be held Friday evening, November 11, and the annual business meeting November 12 at 12:30 P.M.

Additional information may be obtained from the Office of the Secretary-Treasurer, 634 North Grand Avenue, St. Louis 3, Missouri.

THE NEW YORK ACADEMY OF MEDICINE

The New York Academy of Medicine will hold its Twenty-Eighth Annual Graduate Fortnight *October 10–21, 1955*, inclusive, on the subject PROBLEMS OF AGING. The program will consist of 20 evening lectures and 4 evening panel meetings; 6 morning panel meetings; 19 afternoon hospital clinics; and a Scientific Exhibit. Five Community Educational and Recreational Day Centers for the aged will hold open house.

Registration fee, $10.00 for entire program—$6.00 for either week. All inquiries regarding fees and all requests for registration should be addressed to: Secretary, Graduate Fortnight, New York Academy of Medicine, 2 East 103 Street, New York 29, N. Y.